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BRUCELLA ABORTUS INFECTION IN MAN*

A CLINICAL ANALYSIS OF THIRTY-FIVE CASES

BY ALFRED S. GIORDANO, M.D., M.SC., AND R. LLOYD SENSENICH, M.D.
SOUTH BEND, INDIANA

DURING the past three years there has been reported the widespread existence of a disease caused by infection with organisms belonging to the group of Brucella alkaligines. In this group there are several strains, classified according to the animal from which they were originally isolated: the caprine, bovine, porcine, and others. The caprine strain, Brucella melitensis, was first isolated and described by Bruce[1] in 1887. We are indebted to Hughes[2] for a classical clinical description of the disease resulting from this infection, which is characterized by attacks of undulatory pyrexial relapses, profuse sweats, rheumatic pain, and enlarged spleen.

Another strain now known as Brucella abortus was isolated by Bang[3] in 1897 from the placentas of aborting cattle, but it was not thought to be pathogenic for man. Evans[4] later showed the similarity of the cultural and serologic characteristics of Brucella melitensis and Brucella abortus, and suggested that Brucella abortus, like Brucella melitensis, might cause disease in man. This theory was first definitely proved in 1924 by Carpenter,[5] whose experiments fulfilled Koch's postulates. Since his publication many cases of Brucella abortus infection in man have been reported. Confusion has, however, arisen in that the relationship between Brucella abortus and Brucella melitensis has apparently led to the assumption that Brucella abortus infection in man must necessarily follow a clinical course identical with that produced by infection with Brucella melitensis.

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From the South Bend Medical Laboratory.

In 1927, while studying the clinical features of Brucella abortus infection, we[6] presented a preliminary report of a series of seven cases. These patients were under observation for a relatively short period. No attempt was made at the time to classify the cases studied except along the lines generally followed in the consideration of cases of undulant fever due to Brucella melitensis infection.

With opportunity for prolonged observation and the addition of twenty-eight cases to the group studied, the relative infrequency of the typically undulant febrile course raises the question as to whether or not Brucella abortus infection in man tends to follow a clinical course somewhat at variance with that of the disease due to Brucella melitensis. On the basis of additional information gained by more complete study, these variations may now be more definitely described.

INCIDENCE

Sex does not seem to be a factor. In our series there are 20 men and 15 women. The incidence by decades is as follows:

AGE, YEARS	CASES
11 to 20	1
21 to 30	9
31 to 40	13
41 to 50	9
51 to 60	2
61 to 70	1
Total	35

In the total of 35 cases there is a preponderance of infection in the second, third, and fourth decades. The youngest patient is sixteen and the oldest sixty-one years of age. The reports in the literature record no cases in children below eight years of age. This apparent immunity in children parallels that observed in young calves.

Occupation is an etiologic factor only in so far as it provides opportunities for infection: bacteriologists working with these organisms; veterinary surgeons or others engaged in animal husbandry, or those handling the carcasses of infected animals. The incidence of infection in this class is extremely low as compared with those in which infection is traceable to the ingestion of infected milk.

Hardy[7] recently reported an analysis of 125 cases in which 82 patients were engaged in occupations in more or less direct contact with live stock, meats, or dairy products. Among these, 56 were farmers, 8 farmers' wives, 3 stock buyers, 12 packing-house workers, two workers in dairy products, and one a butcher. The remaining 43 were distributed among various occupations and professions. From this analysis the only direct occupational incidence is that of the 12 packing-house employees, and in this group it would have to be proved that raw milk had not been ingested. Certainly one could not classify the incidence among farmers as an occupational disease, unless aborting animals were treated. The high incidence in this class must also be considered from the basis of the consumption of infected milk, just

as among urban dwellers with no contact with infected animals in communities where pasteurization of milk is not practiced.

In our series, 5 patients were farmers, one a veterinary surgeon, one a mechanic who also raised pigs and on occasion handled infected placentas, and the remaining 28 were in urban occupations such as trades and professions, including three physicians. In only one case in this series was contact infection established; this patient was a veterinary surgeon.

RESIDENCE

The incidence of the disease in small communities is usually much greater as there are fewer pasteurization plants, and raw milk is more generally consumed. Of the 35 cases herein reported only six patients lived in towns of more than 50,000. The remaining cases occurred in small communities of less than 20,000, and many of these patients visited relatives owning farms whose herds were found to be infected with Brucella abortus. The importance of Brucella abortus infection as a rural health problem is at once apparent since in all of our cases the patients consumed raw milk.

MODES OF INFECTION

While the modes of infection are variable, occurrence of the disease following the ingestion of milk containing Brucella abortus in cases reported by us, Carpenter,[5] Huddleson,[8] Evans,[9] Kern,[10] Simpson,[11] and others, leaves no doubt as to the manner of infection in these cases.

INCUBATION PERIOD

In only one of our cases could the incubation period be approximately determined. In accidental laboratory infections the incubation periods have varied from seven to twelve days.

MODE OF ONSET

The initial symptoms are as widely variant as is the clinical course. An insidious onset with fatigue, anorexia, low fever, headache, with muscular weakness or joint discomfort is the most common. Chills are usually not the initial symptoms and develop after elevation of temperature is noted. The presence of pharyngitis, slight cough, or bronchitis frequently lead to a mistaken diagnosis. Rarely is the onset more abrupt, and the evidences of sepsis are the first and temporarily overwhelming symptoms. One such patient ran a very brief course with early recovery and without relapse, whereas a rapid malignant course with hyperpyrexia and fatal termination has been reported by Hardy[7] and others.

Arthritis with moderate elevation of temperature may be the first and only manifestation of the disease, or the nervous system may be attacked and the invasion may be characterized by meningeal symptoms or neuritis.

CLINICAL FEATURES

The disease may be acute or chronic, the course in each instance differing not only in duration of illness but in other respects, and varying to such a degree as to be distinctive. One of our patients with chills, sweats, headache,

and fever of septic type, with no tendency to undulation, recovered in two weeks with no subsequent relapse, whereas another patient has for a period of illness of more than ten months had a continual course of chills, sweats, and septic type of fever without undulation or a single remission. In a third case, reported in an earlier communication, the patient suffered an illness of six months' duration characterized throughout by undulating temperature and apyrexial remissions.

The chronic course of the disease has been commonly described in the literature of undulant fever, whereas the acute course has been infrequently discussed, or discussed with the assumption that the apparent short course was accounted for because of an insufficient period of observation and that the illness must have included many such attacks with periods of remission.

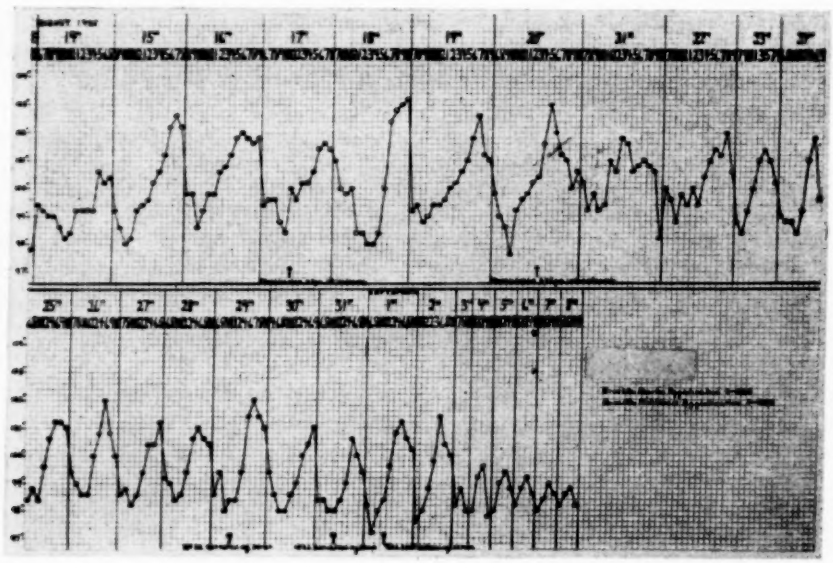

Fig. 1.

As a result of this, the chronic type has been generally accepted as the most frequent and typical. From our observations in Brucella abortus infection and a study of those cases reported in the literature, it is apparent that the cases of acute type are more frequent than the chronic type described, and it is suggested that the tendency to acute or chronic illness may be a differential point between the Brucella abortus and Brucella melitensis infection.

Although the disease due to Brucella abortus presents a widely varied symptomatology and although certain symptoms may increase or diminish in intensity or may be supplanted by other symptoms, there is noted in the cause of illness in most cases a dominance of certain symptoms directly referable to the particular body structures that are being attacked by the disease. For purposes of better comparison and study, therefore, an effort will be made to define more closely, and to classify, cases of this disease on the basis of predominant symptoms and various body tissues attacked. That this classification may be practical and helpful, we have considered under the following

subdivisions all the cases that have come to our attention. The brief histories submitted were chosen as examples typical for the group.

Septic Type.—Into this group we have placed those cases in which the course of the disease has been similar to other infectious diseases in which bacteremia is an underlying condition.

A man, aged thirty-two, a physician, became ill on July 1, 1928. The onset of the disease was acute with chills, fever, profuse perspiration, marked weakness and rapid loss of weight (15 pounds). Headache and backache were distressing and there was pain in both eyes. A slight cough persisted throughout the first ten days of the illness. There was an emotional instability markedly contrary to the man's usual personality. The fever was septic in type reaching 102.6° to 103° F. at six o'clock in the evening and exhibiting marked morning remissions. There was no tendency to undulation. Physical examination revealed no abnormalities aside from an old mitral valvular lesion and tenderness of both testes which developed soon after the onset of this illness. The urine was normal. The hemoglobin was 90 per cent; erythrocytes normal; leucocytes, 4,300; polymorphonuclears 42 per cent; small lymphocytes 47 per cent; large lymphocytes 10 per cent; eosinophiles 1 per cent. Agglutination tests for Bacillus typhosus and Bacillus paratyphosus A and B were negatime. Brucella abortus agglutinated in a dilution of 1:1,280 and Brucella melitensis in a

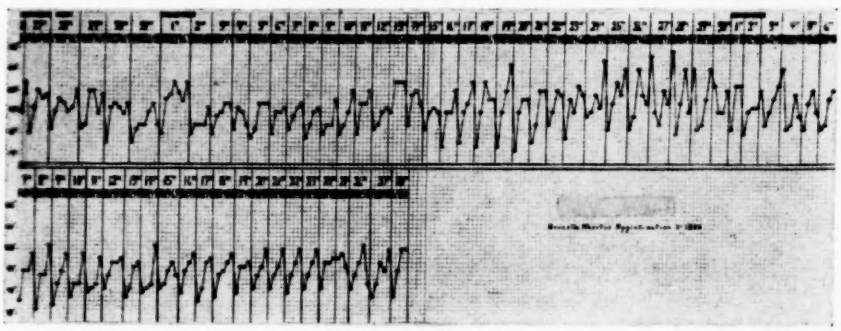

Fig. 2.

dilution of 1:160. The intradermal test was positive although blood cultures remained sterile. The patient was treated with salvarsan without effect. Convalescent serum was given with no immediate results, although after two weeks of this treatment the temperature gradually became normal. Recovery was complete in three months and there has been no relapse.

Arthritic Type.—Into this group we have placed those cases in which dominant symptoms have been referable to the joints. They have varied from painful tender joints in which the involvement may have been periarticular to conditions in which recurrent hydrops was the characteristic manifestation.

A well-nourished boy, aged sixteen, in July, 1926, complained of "rheumatism in both knees" and of profuse sweats. In October, 1926, following an injury, an abscess developed in the right leg below the knee. This was drained surgically and gave no evidence of association with the joint symptoms described. There was no evidence of bone involvement and x-ray examination was negative. Indefinite joint symptoms continued until February, 1927, at which time he suffered an attack of arthritis involving the shoulders, elbows, wrists, hips, knees, ankles and feet. This attack was of short duration but recurrent shifting joint symptoms continued. The attacks were accompanied by fever ranging from 99.5° to 104° F., with chills, profuse sweats, a furred tongue, and some tenderness to pressure over the liver. Physical examination in the interim between attacks did not reveal any abnormal

ities. The arthritic manifestations varied from a moderately tender and painful joint in the milder attacks to a single swollen and extremely painful joint. Despite the most extreme local manifestations in a joint, no redness of the integument was noted. The rapidity with which extreme joint changes developed in an apparently normal joint was remarkable as was the equally rapid disappearance of symptoms; they left no sign of impairment. The urine during one acute attack contained albumin, an occasional granular cast, and a few pus cells in each field (high power). The urine and also the blood count were normal after the acute attack. Examination for malarial parasites was negative. The Wassermann reaction was negative. Agglutination tests for Bacillus typhosus and Bacillus paratyphosus A and B were negative. The blood serum agglutinated Brucella abortus in a dilution of 1:1,280 with both bovine and porcine strains. Blood cultures for Brucella abortus yielded a few colonies of Staphylococcus aureus. The patient recovered after more than a year of illness, and there has not been any evidence of recurrence in sixteen months.

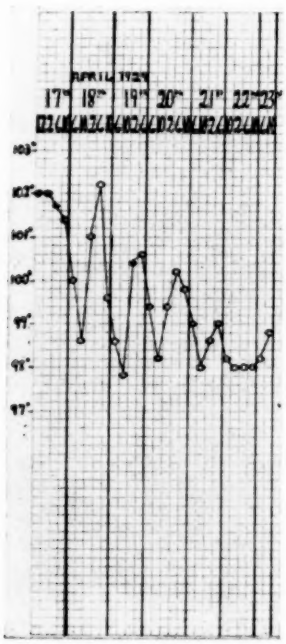

Fig. 3.—Brucella abortus agglutination 1-640; Brucella melitensis agglutination 1-120.

Neurologic Type.—We have limited this classification to those cases in which there was indicated an invasion of the nervous system to such an extent that the resultant clinical manifestations were definitely neurologic in character. We excluded the minor manifestations of nervousness which would generally accompany any infectious disease of this character.

A farmer, aged forty-two, was referred by Dr. J. C. Fleming. The patient, well nourished and previously of good health, became suddenly ill with severe headache, chills, septic fever, and muscular weakness. He was free from arthritic pains, chest or abdominal symptoms and skin manifestations. In the cervical region there was marked tenderness to any pressure or effort at movement and the cervical rigidity suggested meningeal involvement. Patellar reflexes were increased but no other abnormalities were found. The urine and the blood count were normal. Agglutination tests for Bacillus typhosus and Bacillus paratyphosus A and B were negative. Brucella abortus agglutinated in a dilution of 1:320, and Brucella melitensis agglutinated in a dilution of 1:40. The patient recovered completely in two weeks and there has been no recurrence of symptoms.

Visceral Type.—The bacteremia in this disease leads to visceral invasion, and lungs, heart, liver, gall bladder, bowel, spleen, kidneys, uterus, and adnexa have been involved. Abortions have occurred in women infected with Brucella abortus, although we have seen no reports of abortions due to Brucella melitensis. Under this visceral type we have grouped all patients having dominant visceral symptoms.

a. An automobile dealer, forty years of age, a patient of Dr. J. C. Fleming, came under observation on April 24, 1928. His history contained nothing of moment other than an attack of painless jaundice three years prior to this illness. At that time the gall bladder had been drained and improvement had followed. The patient had remained in good health until March 28, 1928, when he noted a loss of appetite, fever and jaundice. The fever was septic in type and gradually rose until it reached 104° F. At this time tenderness over the gall bladder was noted. The surgeon considered cholecystectomy inadvisable at the time, so a cholecystostomy was done. The leucocyte count was 5,000. Agglutination of Bacillus

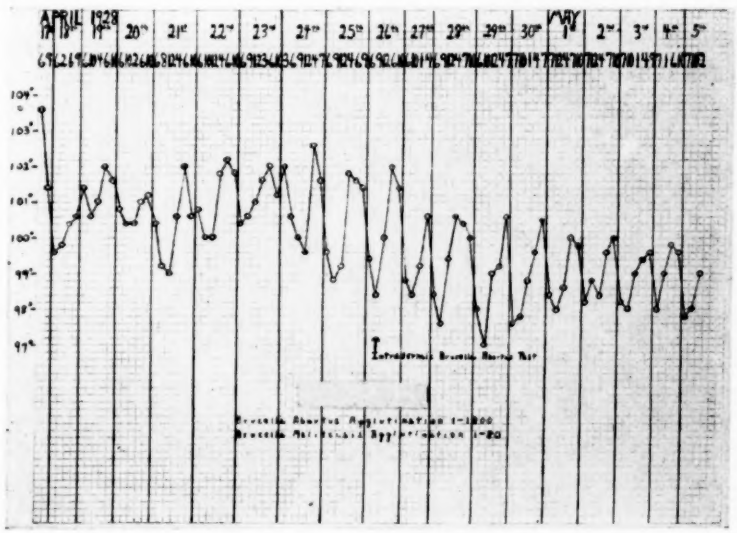

Fig. 4.

typhosus occurred in a dilution of 1:20. Agglutination of Brucella abortus occurred in a dilution of 1:1,800 and Brucella melitensis 1:80. Culture from the bile was made and the organisms injected into two guinea pigs and they became infected with Brucella abortus. The intradermal skin test was positive for Brucella abortus. There was no immediate improvement after operation. Fever and sweats continued for two weeks, after which gradual recovery took place. Jaundice disappeared after cessation of fever. The course of this illness was of about two and a half months' duration. There has been no recurrence.

b. The brief history available in this case is strongly suggestive. The patient, a woman who had been married for ten years, had always been in good health prior to the present illness. In the first six years following her marriage she had given birth to five children. All pregnancies had been normal and labors normal and uncomplicated. All of the children are living and well. During the past four years she has suffered from marked weakness and pain in the back and legs. Headaches have been so severe that it has been almost impossible for her to be up and around. The patient has had frequent attacks of chills and fever which have persisted for periods of two or three months. During this period of four years she aborted four times at about the fifth month of pregnancy. History and physical examination failed to reveal any cause for the repeated abortions. The urine was normal.

Blood examinations for malaria and the Wassermann reaction were negative. Brucella abortus agglutinated in a dilution of 1:160.

c. A man aged thirty-five, a physician, previously in good health, became ill in November, 1928, with fever, chills, sweats, backache, epigastric pain and vomiting. There was marked muscular weakness and loss of weight. The temperature was remittent and continued for four months. On physical examination the liver was found to be markedly enlarged and tender. There was no jaundice. The heart and lungs were normal. There was no evidence of genitourinary disease or orchitis. The urine was normal. The erythrocytes were normal in number, and leucocytes numbered 6,000 with a normal differential count. Agglutination tests for Bacillus typhosus and Bacillus paratyphosus A and B were negative. Brucella abortus agglutinated in a dilution of 1:1,280. The enlargement of the liver continued for more than four months. Recovery followed at the end of five months and there has been no relapse. The patient states that he has had no residual asthenia or any other symptoms.

Glandular Type.—This type is undoubtedly less common than those before described, and includes those cases in which regional glands have been infected by reason of proximity to some point into which a large number of virulent organisms have gained entrance.

A man aged forty, a veterinary surgeon, presented nothing of importance in the history other than acute arthritis at the age of twenty-two. This arthritis had been confined

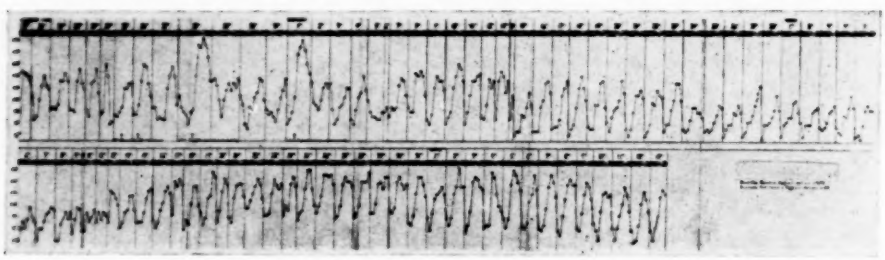

Fig. 5.

to the feet, knees and hips. There had been occasional recurrent attacks, though not severe or disabling. He was then a student in the department of agriculture of a large university and was specializing in animal husbandry. He later completed a course in veterinary surgery and in 1925 came under observation because of enlarged tender and painful axillary glands, and attacks of chills, fever, sweats and backache. The attacks of fever, chills and sweats were always an accompaniment of the swollen axillary glands and in periods of recession of the glandular inflammation the acute symptoms were absent. Backache and general malaise, however, frequently continued for long periods and the axillary glands were palpable. Hospital observation and laboratory examination failed at that time to reveal the character of the infection. Following a period of rest the patient's condition was markedly improved. In May, 1928, after a period of unusually distressing backache, agglutination tests were made. Brucella abortus did not agglutinate. The intradermal test was positive for both Brucella melitensis and Brucella abortus. More recently there has been an acute attack with chills, high fever, sweats and extremely distressing headache and backache. The axillary glands were again markedly swollen, painful and tender to pressure. As a veterinary surgeon the patient is often called on to care for aborting animals. In this work he has frequently acquired infections on the hands and arms, and has recently observed that the exposure of the unbroken skin of his arm to abortus-infected animal discharges will cause the appearance of an eruption and if the skin is not carefully cleansed there may be a recurrence of glandular swelling and acute symptoms. Considering the university course in animal husbandry, it is impossible to determine the date of the original infection or number of possible reinfections because of the frequent exposure to infected animals throughout a period of nearly twenty years.

SYMPTOMS: THEIR FREQUENCY AND CHARACTER REGARDLESS OF TYPE

Fever.—Of the individual symptoms, fever is the most constantly present and the most variable in character. Owing to the bacteremia of this disease, the fever in most cases is somewhat similar to that associated with other septic conditions. The undulant type of fever is the most striking but more often a feature of the chronic cases. The temperature for a period of from seven to fourteen days rises by step-like gradations, each day reaching a higher point and exhibiting a morning remission to, or near, normal. (Fig. 6.) The fever having reached a daily peak of 103° or 104° F. with a morning remission of two or three degrees, begins to exhibit the undulatory phenomena as follows: The daily peak and low point in remission each day occur at a higher point than the preceding day until the crest of the wave is outlined, whereupon for an equal number of days the temperature in like manner recedes to a lower level each day. Such a temperature chart viewed over a period of weeks exhibits a series of undulations each requiring the same number of days for completion.

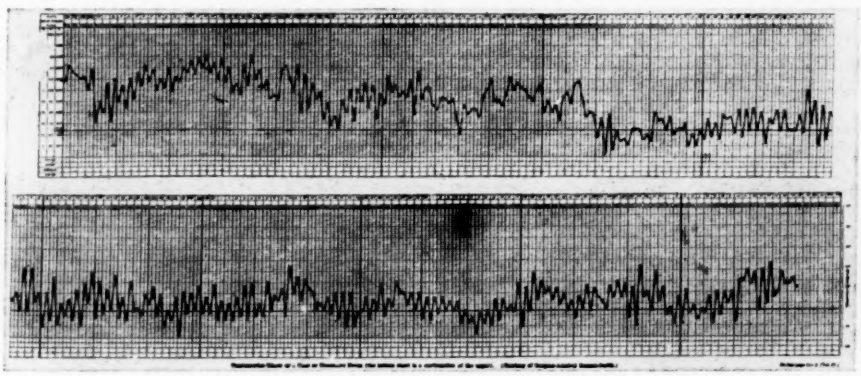

Fig. 6.

In one of our cases the undulations covered eight days, while in another one the cycle was completed in four days. In the melitensis infection each cycle may cover a period of three weeks or more. Hughes,[2] who first suggested undulant fever in Brucella melitensis infection as descriptive of this phenomenon, noted the variable length of the undulations in different cases but pointed out the striking constancy of the undulatory pattern once it was established in a given individual. The period of typical undulation is variable in length. In one of our cases it existed for approximately four weeks while in another this type of fever persisted for many months. There is a gradual tendency to lower levels with apyrexial intermissions followed by short periods of relapse with irregular fever, and change to the intermittent type. This in one of our cases persisted for a period of six months. This undulant febrile record is parallel to that of undulant fever due to Brucella melitensis. Such a case was reported in our first series. With opportunity for study of temperature records of many more patients, we are now rarely finding this typical febrile pattern. We are seeing instead a tendency to constant septic fever with moderate daily remissions, variable in course from a period of two weeks to ten

months, without tendency to undulation or apyrexial intervals. This, too, has recently been pointed out by Hardy.[7]

It is also noted that after improvement has begun there is steady and fairly rapid progress to an apparently complete restoration of health without sequelae or recurrence. Inasmuch as our studies are of Brucella abortus infections and our findings are apparently at variance with the more frequently reported undulant febrile course in Brucella melitensis and although mild and intermittent types of that disease are also reported, it is suggested that the Brucella abortus infection may tend to the production of an illness of shorter duration, at the same time differing from the melitensis infection in febrile course. In view of the difficulty in diagnosis of the short acute illness, it is probable that with an awakened interest on the part of physicians and increased facilities for laboratory diagnostic tests for this disease, that a still larger proportion of cases running a short course will be discovered.

Chills.—Chills are equally as constant as is fever. They are present early in the illness and tend to persist, although they occur less frequently later. They vary in intensity from a mild chilliness to violent rigor and are usually followed by a rise in temperature, but may occur only as a distressing chilliness while the temperature is elevated. Recurrence at the same time daily throughout a long period of time is frequently noted.

Sweats.—Sweats are associated with the periods of chills and hyperpyrexia. They may be mild or extremely profuse and may occur independently of chills. These sweats may be of noticeably offensive odor.

Headache.—Headache, especially in the early stages of the illness, is constant and extremely distressing. It is more frequently occipital and the pain extends downward through the cervical region and then is diffused over the shoulders and upper portion of the back. The severe pain has a tendency to regular periodicity, recurring at the same time each day, usually with increases in temperature. Later in the illness and in the presence of lower temperature, it is lessened or disappears.

Backache.—Backache is at first usually related to the occipital headache as before described, and generally diminishes in intensity with it. It may, however, persist independently or even recur after the febrile period. It is frequently confined to the lumbar region.

Arthralgia.—Arthralgia is fairly constant and the onset is usually early in the illness. It is generally lessened with disappearance of the acute septic symptoms. It may, however, persist throughout the illness. It is variable from a mild discomfort to a persistent pain in the smaller joints likened to the pain of a crushing injury. Less frequently is there discomfort about the larger joints. The pain is apparently due to periarticular neuritis.

Arthritis.—Arthritis is a distressing and frequently disabling manifestation of this disease. It may develop early or late in the course, or may exist as the dominant symptom throughout. In some cases it has apparently been the only manifestation of the disease. All the joints may be involved, separately or together, or it may shift rapidly from joint to joint. There may be a definite periodicity of return as in one of our cases, in which acute attacks recurred at regular intervals for many months. The joints involved may be

painful and tender, but even though they are swollen there is seldom redness. There may be marked hydrops which recurs at definite intervals of days or weeks, develops rapidly and recedes promptly as in a case reported by us and one reported by Baker.[12] In our case cultures of the organism were obtained from fluid aspirated from the involved joint. No permanent impairment of joint function followed.

Neuritis.—Neuritis, like arthritis, may occur at any stage of the illness, and may be of variable duration or the only symptom of the disease. It may be evidenced by pain in any locality and is probably responsible for most of the pain about joints and in the back, as was described. There may be a noticeable cutaneous hyperesthesia, but no areas of anesthesia have been demonstrated in our cases. An unusual type of discomfort is that described by many of our patients as "eye-ache." It is not due to photophobia, is not caused or accompanied by visual disorders, and may be present at any time. Persistent or recurring sciatica is frequently due to this infection.

Other nervous manifestations of this disease are present almost without exception. Asthenia is an early and constant symptom and frequently persists for a long time after relief from all other symptoms. Depression, irritability, or anxiety neurosis are frequent variations in the mental condition of the patient. Even in the presence of high fever there was never the dullness or apathy, such as commonly characterizes the typhoid state. Exaggerated reflexes and evidence of tension are common. Tremor occurs and in one of our patients the whole body was affected. This was so pronounced that intelligible speech was almost impossible. A complete recovery followed. Insomnia is frequently a troublesome symptom. Psychic and functional nervous disorders are the most common sequelae.

Gastrointestinal Symptoms.—Anorexia is frequently the first symptom noted, but there is usually a return of appetite as the acute febrile stage subsides. Aside from anorexia and constipation, gastrointestinal symptoms are not common. Nausea and vomiting occur rarely. Epigastric distress is an occasional symptom. In two cases cholecystitis was suspected, and in one instance the gall bladder was drained but without relief of symptoms. There is nothing distinctive about the appearance of the tongue.

Orchitis and Oophoritis.—Orchitis and oophoritis are frequently present. They usually occur during the febrile periods of the disease. Orchitis was a persistent and distressing symptom in two of our cases in which the clinical course had been most mild. There is tenderness and perhaps some swelling. In our cases there was no history of previous disease. This symptom may persist after recovery from other acute symptoms.

Skin Manifestations.—Unusual skin lesions were seen in six of our patients: In two instances these occurred as tubercle-like elevations on the dorsal surface of the fingers and hands. In others it also involved the face and body. They appeared as small maculae and persisted for a few days. In one patient these macular lesions were accompanied by an intense itching that lasted for two weeks.

Splenic Symptoms.—The spleen was palpable and tender in two of the cases in our series. Notable enlargement of the spleen, so commonly observed

in Brucella melitensis infection as to be regarded as a cardinal symptom, was observed only once and that was in a patient whose blood agglutinated melitensis only.

Hepatic Symptoms.—Definite enlargement of the liver occurred only twice in our series. In one case it was associated with vomiting. In three cases under our observation there were symptoms suggestive of gall bladder disease, and in one case there was jaundice. A cholecystostomy was done on the jaundiced patient and in this case Brucella abortus was obtained on culture from the bile. The jaundice continued after the operation and until the febrile stage had passed.

Renal Symptoms.—The kidneys may be impaired. A moderate amount of albumin and a few casts were found in some of our cases, although considering the character of the illness such changes were remarkably rare. The organism was not found in urine cultures.

Respiratory Symptoms.—Pharyngitis is frequently an initial complaint. Pulmonary symptoms are common. Bronchitis varying in intensity may come early in the disease and persist throughout. Pneumonia and pleurisy with effusion may occur. Diagnosis of pulmonary tuberculosis was made in one of our cases.

Cardiac Symptoms.—Heart manifestations due to Brucella abortus are fortunately not common. The pulse rate is accelerated and in the absence of fever the case might be mistaken for one of hyperthyroidism. Rhythm and valve sounds are not often altered unless this occurs in previous disease. However, one death from endocarditis due to this organism has been reported by Saphir.[13]

Affection of Lymphatic Glands.—Lymphatic gland involvement is usually not demonstrable in this disease, except in the cases of regional glands which have become infected by reason of close proximity to the point of introduction of an overwhelming number of virulent organisms. This condition exists in one of our patients, a veterinary surgeon, whose axillary glands are enlarged and tender, due probably to repeated infection from operative wounds sustained in handling infected cattle. The demonstrable changes in these glands vary with the various stages of activity of his infection as evidenced by clinical phenomena and blood examination. No doubt infected lymph glands frequently harbor this organism throughout protracted periods. Carpenter has repeatedly cultured organisms from tonsils removed at operation.

DIAGNOSIS

Undulant fever as regards diagnosis on the basis of clinical observation alone requires differentiation from many lesions due to infection. Typhoid fever, malaria, tuberculosis or other respiratory infections, and sinusitis, rheumatism, cholecystitis or liver abscess, meningitis or any pyogenic infection must be considered. The recent report of cases of leishmaniasis in this country might rarely make it necessary to consider the possibility of this disease. The differential diagnosis from each of the above-named conditions cannot be discussed in detail in this paper.

The history as to probable ingestion of infected milk or contact with infected animals is frequently strongly suggestive. The chills, fever, sweats, muscle or joint discomfort, headache and evidence of involvement of the nervous system without gastrointestinal symptoms, rose spots, hemorrhage or demonstrable focal infection furnish valuable differential evidence, although all these variations may occur. A persistently chronic course uninfluenced by quinine or salicylates adds therapeutic evidence if no laboratory facilities are available. Definite diagnosis must be made by the clinical pathologist.

CLINICAL PATHOLOGY

The leucocyte count early in the disease is usually diminished. The lowest count was 3,800 and the highest was 14,000. Later in the course the count varies from 6,000 to 10,000. The differential count is usually normal or there may be a slight increase in mononuclears, but this, too, is a variable factor. The erythrocyte count and the hemoglobin have always been well within normal limits. The urine is essentially negative. Blood cultures have been extremely difficult to obtain in this series, but of twenty cultures only two were demonstrated as positive. On the second attempt in one case, a few colonies were grown on Huddleson's liver agar media in 10 per cent CO_2 atmosphere, although the guinea pig inoculations were negative. In another case the guinea pig inoculation was positive, and the culture negative. It appears that positive cultures are more likely to be obtained at the height of temperature.

AGGLUTINATION TEST

There is no dispute that the agglutination reaction is the most valuable sign in the diagnosis of undulant fever. In the majority of instances, agglutinins appear early in the course of the disease. There are several reports in the literature of patients with no agglutinins in the blood serum, but with positive blood cultures. Tramontano in 65 confirmed cases of undulant fever found nine serums which failed to agglutinate. Carpenter[14] isolated the organism in five cases of undulant fever, in only three of which agglutinins were present. Our cases suggest this possibility but further investigation of this point is indicated.

The agglutinin titer varies from 1:5 to 1:20,000. Serums agglutinating Brucella melitensis or Brucella abortus in a dilution of 1:40 or higher, it is generally agreed, indicate active infection with these organisms, but the significance of agglutination in low titer is still open to discussion. It has been suggested that absorption of agglutinins from ingested milk may take place. This seems a little difficult to understand in view of the original low agglutinin content of milk. It is also known that absorbed agglutinins are excreted rapidly, yet in our experience some patients with agglutinins in the blood had not consumed milk for months previous to the test. Others regard these agglutinations as nonspecific. Evans believes the agglutinins arise as a specific response to infection with Brucella abortus ingested with cow's milk, although such an infection may not result in illness. A study of our series seems to substantiate this conclusion.

Agglutinins may result from an old infection from which the patient has entirely recovered. They may be present in the blood of a carrier. This assumes a focus of infection and constant agglutinin production to combat the organisms released, or it may be the response to a recent active infection. Instances of the carrier state have been cited by Shaw.[15] He was able to isolate Brucella melitensis from the blood of ten Maltese dockyard employees, none of whom exhibited symptoms. Vaccaro also isolated the organism from the urine of a patient who was apparently well but whose blood contained agglutinins for Brucella melitensis.

In the present study most of the serums from actively ill patients agglutinated in dilutions higher than 1:160. On the other hand, in two active cases the serum did not agglutinate in more than 1:10 dilution. In general it can be stated that the agglutinin bears no relation to the activity of the illness and the agglutinins remain in the blood for more than ten years. Our own study of this problem has convinced us of the necessity of using several strains of this organism in the routine test. One patient who gave a history of at least three years of recurring attacks of fever, chills, headache, and excruciating lumbar pain, had been under medical supervision for a long period. Preliminary agglutination with Brucella abortus (No. 80, Meyer) was negative. Because of the dominant symptoms of this disease, seven other strains were used, including one paramelitensis and two other melitensis strains of ascertained origin. This subsequently showed complete agglutination of Brucella melitensis in dilutions up to 1:1,800 with all melitensis strains. Another complication that is occasionally encountered is the so-called zone phenomena or preagglutination zone in which agglutinations occur only in high dilutions. Cross agglutinations with Bacterium tularense were reported recently by Francis and Evans,[16] but the difference in the titer clears the diagnosis.

AGGLUTINATION TITER AND PROGNOSIS

On the basis of the present data, as has already been stated, there is no relationship between the titer of agglutinins and the severity of the infection. Usually, however, the titer gradually falls as the patient recovers, but a proportionately high titer is maintained for at least one year or more.

IMMUNITY

At present there are no available data on the subject of immunity, but with present knowledge one might infer that natural immunity is present in many cases.

DERMAL TEST

With the recognition of serologically negative cases of Brucella abortus infection an attempt has been made to develop a skin reaction test. As previously reported,[17] this test yields specific skin reactions in known positive and negative cases, and promises to be an additional aid in the diagnosis. With this point in view, the test is worthy of further investigation over a larger series of cases.

TREATMENT

Rest in bed with symptomatic treatment during the period of acute manifestations is the treatment indicated, and has in our series given results equal to any specific therapy thus far advanced. Rest should be maintained until the temperature has continued normal for at least a week and even then relapse may follow physical exercise. Five patients in our series resumed their usual duties without apparent ill effect after a few days in bed. Chemotherapy is suggested as a possible therapeutic procedure, but in view of the low mortality in this disease it should be determined in advance that the administration of a given chemical agent is not attended by undue risk. Reports from the use of mercurochrome are conflicting and its use does not seem to be justified on the basis of present information. Neutral acriflavin was administered to one patient in the third week of illness, and a severe reaction immediately occurred. Disappearance of fever followed in a few days, although a relapse occurred after eight months of apparently normal health. Administration of this preparation to another patient had to be discontinued because of the violent reaction when only 5 c.c. of a 1 per cent solution had been given. No improvement followed.

The efficacy of foreign protein therapy has been reported in the literature. We have used intramuscular injection of milk, and intravenous administration of typhoid vaccine but without influencing the course of the disease. Baker[12] has reported beneficial results in a case of intermittent hydrarthrosis treated with convalescent serum, but a relapse is said to have occurred later. This treatment was also given by Moss in a case of undulant fever but without permanent results. One of our patients having been treated with salvarsan without results was given convalescent serum. There was no immediate effect, but improvement began a week later and continued to complete recovery. Specific vaccine therapy has been used in Europe in treatment of Brucella melitensis infections, and it seems promising.

There is as yet no general agreement as to results.

SUMMARY

A clinical study of thirty-five patients infected with Brucella abortus is herein presented in which an effort is made to more closely define the clinical course on the basis of predominance of symptoms as to particular body structures involved.

Results of these observations suggest that Brucella abortus infection tends to run a clinical course somewhat at variance from that of Brucella melitensis infection.

It is apparent that the illness more frequently runs an acute course, septic in type, without characteristic pyrexial undulations or relapses and terminates in recovery without notable sequelae.

The illness, if protracted, presents a clinical picture sufficiently distinctive as to make clinical recognition possible, but early diagnosis must depend on bacteriologic and serologic studies.

In the light of present knowledge, symptomatic treatment with rest in bed is the treatment of choice.

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THE LABORATORY DIAGNOSIS OF UNDULANT FEVER*

By Charles M. Carpenter, Ph.D., and Ruth A. Boak, Ph.D., Ithaca, N. Y.

THE recognition during the last five years of the frequent occurrence of undulant fever in the United States has prompted many laboratories to devote considerable time to the study of Brucella abortus and Brucella melitensis infections in man. There is not much doubt that both organisms now classed as two species originated from one source. Apparently, Br. abortus has adapted itself chiefly to cattle while Br. melitensis has been able to propagate itself more advantageously among goats. Incidentally, both organisms have become invasive for man and produce symptoms which cannot readily be differentiated. Aside from eliminating the sources of these infections for man, the differentiation is not important, because the diagnosis and treatment, as well as the course of disease caused by the two agents, are the same.

The biologic characters of the two organisms are so similar they can be differentiated only by studying the antibodies produced by each. There may be variations in their atmospheric requirements under artificial cultivation, differences in their degrees of pathogenicity for experimental animals, in their antigenic properties, and in their morphology as well as in their natural habitats, but these cannot always be relied upon. Several investigators have described methods for identifying the two types, but in our experience, with the exception of the agglutinin absorption test, these cannot be depended upon absolutely. Many quantitative differences can be observed when a large number of melitensis and abortus cultures are examined, but no infallible qualitative test, with the above exception, has been developed. A few cultures of Br. abortus are always found that show a characteristic of so-called melitensis and vice versa.

The agglutinin absorption test requires experience and may fail if employed by one who is not accustomed to the technic or by individuals not familiar with the peculiar cultural characteristics of both species. The test depends upon the use of authentic cultures of Br. abortus and of Br. melitensis or of antiserums prepared from them. We now know that both microorganisms may be isolated from man and that they may be recovered from various species of animals. In our studies we have employed Br. abortus, "80," isolated from certified milk by Dr. K. F. Meyer[1] and Br. melitensis, "428," also obtained by Dr. Meyer from Dr. E. Sergent, Institut Pasteur, D'Algerie, Tunis. We know that the antibodies produced by these two cultures are different but are we safe in assuming that they have been named correctly? However, such assumptions must be made because it is necessary to have some foundation upon which future work may be based.

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From the Department of Comparative Pathology and Bacteriology, Cornell University, Ithaca, New York.

By the use of the agglutinin absorption test, evidence has been accumulated to show that the majority of cases of undulant fever in the United States are due to Br. abortus. Because goats have been eliminated as a possible source of infection in most of the reported cases in this country, circumstantial evidence has been presented incriminating cattle, or swine that may have been infected from contact with cattle. It has been well demonstrated that cow's milk is the chief source of infection.[2]

The cardinal symptoms of undulant fever are general malaise, fever, night sweats, enlarged spleen and perhaps a general lymphadentitis. There usually occurs a slow pulse incompatible with the increase in temperature. If these symptoms continue for many weeks there is a loss of weight and a secondary anemia. The infection may localize in the joints or in the genital tract. Unless one has had considerable clinical experience or does not consider undulant fever in his differential diagnosis, it is readily seen how such symptoms may be ascribed to other infectious agents which produce a similar syndrome. Accumulation of information concerning the presence of undulant fever in this country, as well as a review of the literature on the subject, shows clearly that the majority of diagnoses have been made by those engaged in laboratory work. It is evident that during recent months, the trend has been changing and clinicians are requesting laboratories to confirm their diagnoses.

The various laboratory procedures of assistance in establishing a positive diagnosis of undulant fever are as follows:

1. The agglutination test.
2. The complement-fixation test.
3. The bacteriologic examination of blood.
4. The agglutinin absorption test.
5. The blood count.

It is extremely important to collect fifteen or twenty c.c., or even a greater amount, of blood from the patient if possible, because usually there are only a very few organisms present in the blood stream and several tubes and plates must be inoculated because it is necessary to incubate the cultures under different atmospheric conditions. Enough blood should be available for guinea pig injection. The blood for cultures should be obtained in the early course of the disease at a time when the temperature of the patient is highest. We are of the opinion that the reason for so many failures to isolate Br. abortus from the blood is that an attempt to recover this organism is not made until a search for every other infection has been completed. Anticoagulants may be used but there is a possibility that they may injure any organisms that are present. We have in several instances recovered Br. abortus from samples of blood that were free from lithium oxalate and sodium citrate, while specimens that contained them developed no growth. We find as a rule that serum from coagulated blood is more satisfactory for serologic work than plasma from a sample to which some anticoagulant has been added.

THE AGGLUTINATION TEST

The agglutination test is no doubt the simplest and most satisfactory method of ascertaining whether or not a patient has undulant fever. One must keep in mind, however, that it has limitations and that frequently no agglutinins are present in the serum of patients suffering from the disease. Six per cent of the cases we have studied have fallen in this group. On the other hand, when the agglutinins have been formed, they usually remain in the blood a long time after the symptoms have subsided. We have tested the blood serum from three patients monthly for approximately two years, and they still show comparatively high titers. One serum agglutinates the abortus antigen when diluted 1-45, while the other two show agglutination when diluted 1-405 and 1-1215. Quite frequently a serum is observed that shows a marked prezone, namely, the lowest dilutions of the serum which will not agglutinate the antigen, while perfect agglutination is observed in the serum diluted 1-400 or above this point. Therefore it is important to make a series of dilutions beyond this prezone to eliminate the possibility of reporting a positive serum negatively.

The technic of the agglutination test is identical with the usual technic employed for the Widal test. The use of dried blood, however, is quite unsatisfactory. We prefer to use the macroscopic tube test. In our serologic work we have employed an automatic pipetting syringe, known as a rheometer in European laboratories, to avoid the possibility of laboratory infection.[3] Our observations indicate that a living antigen standardized to give a reading of 3.5 cm. on the Gates apparatus is most satisfactory.[4] We dilute the serum directly in the antigen which eliminates adding the salt solution to the tubes and diluting the serum in the salt solution. The tubes are then incubated for eight hours after which time readings are made. They are then set in an ice box for twenty-four hours when they are observed a second time for any delayed reaction. Because abortus agglutinins are usually reciprocal with those produced by Br. melitensis and occasionally with those produced by Bact. tularense, the serum should be set up also with antigens prepared from these two organisms. It is impossible to designate any specific serum titer for a positive diagnosis of undulant fever. Twelve of fifty serums from cases of undulant fever had a titer of 1-400 while the remainder showed titers above and below this dilution, from no agglutinins to a titer of 1-32,805. We have observed severe cases of undulant fever with maximum titers of 1-15 and 1-30.

THE COMPLEMENT-FIXATION TEST

The complement-fixation test has no distinct advantage over the agglutination test. It is more complicated and often serums are found to be anticomplementary but still satisfactory for the agglutination test. The technic is identical with that of the standard Wassermann test, except that an abortus antigen is used. King[5] states that in many serums he has been able to get complement fixation before he could demonstrate agglutinins.

THE BLOOD CULTURE

Due to the variability of the atmospheric requirements of various strains of Br. abortus, cultures should be made by several different methods to iso-

late the organism successfully. Twenty c.c. of blood are collected from the patient and placed in a sterile tube, if it is not convenient to have the medium available for immediate use. As previously stated, we discourage the use of anticoagulants, although specimens of blood made outside of the laboratory may be cultured more easily if the blood has not coagulated. Coagulated samples must be broken up before satisfactory cultures can be made. Any meat infusion agar (preferably made from liver) with a P_H of 6.8 or 7.00 is satisfactory. Four tubes of slant agar may be inoculated and two plates may be poured, to each of which 2 c.c. of blood is added. The serum is collected from the remainder of the sample for serologic work and the clot or cells are injected into two guinea pigs, subcutaneously or intraperitoneally. Two of the slants and one of the plates are incubated in a jar in which 15 per cent of the air has been replaced by 10 per cent carbon dioxide. One of the slants is sealed with paraffin or sealing wax and placed in the incubator under normal atmospheric conditions with the unsealed slant and the other plate. If no growth is observed in seventy-two hours, the blood on the slant agar tubes should be smeared over the surface of the agar and reincubated. Cultures should not be discarded for twenty days, for growth of many of the strains of Br. abortus develops very slowly and in one instance we failed to get a growth until after eighteen days. Recently we have been placing one or two c.c. of blood in 10 or 12 c.c. of liver bouillon, as suggested by Kristensen,[6] who reports that he has been able to recover the organism in about 65 per cent of his cases. We have been more successful when we incubated the blood in the bouillon for seventy-two hours and then injected a guinea pig with 3 or 4 c.c. of the blood-bouillon mixture.

ANIMAL INOCULATION

We consider the guinea pig the most suitable laboratory animal for use in recovering pure cultures of Br. abortus from blood, tissues and milk; although Hagan[7] has demonstrated that mice may be used and that the organism may be recovered from them in a much shorter interval of time than is required in the case of the former. The subcutaneous injection of body fluids and tissues is safer than the intraperitoneal method, but fresh blood from patients may be injected by the latter method without danger to the animal. Theoretically the guinea pig should become infected more quickly by this method, providing the blood has Br. abortus in it. The guinea pigs may be autopsied four or five weeks after their injection, although extensive lesions of the infection are not produced in such a short interval of time except in cases of very virulent strains. Usually two or three months are required for the infection to cause death and occasionally some guinea pigs recover from the disease if they are infected with cultures of low virulence.

After the guinea pig has died or has been sacrificed, the spleen is aseptically removed and placed in sterile Petri dishes for culture. A sample of blood for the agglutination and agglutinin absorption tests should be collected from the guinea pig's heart. A gross examination for abortus lesions in the liver, lymph nodes, reproductive organs, kidneys, and joints should be carefully made. The liver, lymph nodes, or abscesses may likewise be cultured but this is not necessary in a routine examination because the spleen is the

most suitable tissue from which to recover Br. abortus. Bits of the spleen, the size of a pea, are planted on suitable media and incubated, as are the blood cultures, in jars containing 10 per cent carbon dioxide. If no growth is observed in three or four days after incubation, it is well to smear the pieces of spleen over the surface of the media and reincubate the cultures. The identification of the culture recovered and of the antibodies in the serum is made as described in other sections of this report.

IDENTIFICATION OF CULTURES OF BRUCELLA ABORTUS

If bacterial colonies appear on the poured plates or a growth on the tubes, they are picked and transferred to nutrient agar slants to which sterile horse serum has been added. They are then incubated at 37.5° C. under the atmospheric condition which grew them best. After a suitable growth is obtained, the various 1 per cent carbohydrate media are inoculated. A broth culture is likewise inoculated to be examined for motility. Smears from colonies should be made and gram stained, and any abortus bacilli should appear as short gram-negative rods or coccobacilli. It is to be remembered that the morphology of these two organisms is variable and that they have a pleomorphic tendency. The first few generations may show organisms that are almost spherical. On the other hand, we have observed cultures, smears from which showed gram-negative rods that seemed too long possibly to be Br. melitensis or Br. abortus. An antigen is prepared from the growth and an agglutination test is made with an antiabortus serum. If the organisms are Br. abortus or Br. melitensis, it should not ferment any of the carbohydrates, should be nonmotile and should be agglutinated by the antiserum. An agglutinin absorption test is then made to determine whether the organism recovered is Br. abortus or Br. melitensis. This is accomplished by incubating an antiabortus and an antimelitensis serum with large numbers of the unknown organism to absorb the antibodies in the serum specific for the organism.

THE AGGLUTININ ABSORPTION TEST

When the serum from a patient agglutinates both Br. abortus and Br. melitensis, it is necessary to employ the agglutinin absorption test to determine which organism is responsible for the infection. The technic that we have used is as follows: Cultures of Br. abortus (80) and Br. melitensis (428) are grown on the surface of infusion agar in Blake bottles. After forty-eight or seventy-two hours' incubation at 37.5° C., the growth is washed off with a small amount of a physiologic salt solution and centrifuged for one hour in graduated centrifuge tubes at 2000 R.P.M. The supernatant fluid is poured off and about 0.4 c.c. of the packed-down organisms are allowed to remain in the tubes. To obtain this amount may require removal of part of the substrate or the addition of more organisms and a second centrifuging. One c.c. of a 1-5 diluted serum to be tested is placed in each of two centrifuge tubes, one containing 0.4 c.c. Br. abortus organisms, and the other 0.4 c.c. Br. melitensis organisms. If the serum has a low titer, it should not be diluted. Four-tenths of a c.c. of the organisms are a very satisfactory amount for a serum with a titer of from 1-400 to 1-1200, a serum with a higher titer should be diluted ten or twenty times. The serum and organisms are thoroughly mixed, incubated at 37.5° C. for one hour, and then refrigerated overnight. The next morning

the serum and organisms are again centrifuged for one-half hour at high speed. The absorbed serum is removed and set up with antigens prepared from the same cultures of Br. abortus and Br. melitensis as were employed for the absorption of the antibodies in the unknown serum. Agglutination of the abortus antigen caused by a serum absorbed by Br. melitensis indicates that the agglutinins are specific for Br. abortus. However, if agglutination of the melitensis antigen was caused by the serum absorbed by Br. abortus, the agglutinins are specific for Br. melitensis. Brucella melitensis will remove some of the agglutinins from an antiabortus serum and Br. abortus will remove some of the agglutinins from an antimelitensis serum, while each will remove all of the agglutinins from its specific antiserum. The method for identifying unknown cultures by agglutinin absorption is the same as above, except that one must have available known antiabortus and antimelitensis serums. Large amounts of the unknown culture are grown in Blake bottles, harvested and then incubated with the known antiserums.

THE BLOOD COUNT

The blood count is of great assistance in establishing a diagnosis of undulant fever. In very mild cases the blood picture is not changed, but if the symptoms of the disease persist for several weeks, marked changes are observed which cannot be considered specific for undulant fever but furnish corroborative information that aids in making a diagnosis.

An extensive series of blood counts on twenty patients with undulant fever showed a secondary anemia and a leucopenia, with a relative and absolute lymphocytosis as constant changes. The percentage of hemoglobin often drops to 60 per cent and even to 50 per cent, as determined by Sahli's method in severe prolonged cases of the disease. The red cells in one case dropped from normal to 2,872,000 while the average low red cell count was approximately 3,600,000 per c.mm. The lowest white cell count was 2,400 cell per c.mm. while the average was 4200 cells per c.mm. The most marked change in the blood picture is seen in the differential count. The highest percentage of lymphocytes observed was 78 per cent with 22 per cent polymorphonuclear neutrophiles. The average number of lymphocytes was approximately 45 per cent. Some reports have stated that there is a marked increase in the percentage of large monocytes. Our data do not show such evidence. In two cases 24 per cent and 25 per cent large monocytes were counted respectively. One case had been diagnosed as infectious mononucleosis. Of course there is a possibility of both diseases occurring at the same time in one individual. In the majority of cases studied the percentage of large monocytes has remained practically normal. With the increase in the number of lymphocytes, the polymorphonuclear neutrophiles have decreased proportionally. In one instance of a fatal subacute endocarditis due to Br. abortus the white cell count was 11,600 with 86 per cent neutrophiles.

DISCUSSION AND SUMMARY

It is evident that many cases of undulant fever have been diagnosed as other infections in the past, but now the tendency is to base a diagnosis of this disease upon too little evidence. Unless the symptoms are very typical of undulant fever, agglutination of the abortus antigen by the patient's serum is

not a safe criterion from which to draw conclusions, because of the fact that in many cases the antibodies remain in the blood stream months and even years after the symptoms have subsided. However, the fact that certain patients show no antibodies is a limitation of the agglutination test that must not be forgotten. With our present knowledge of cultivating Br. abortus from human blood, its isolation in every case seems impossible, but a positive blood culture gives the most reliable information. There is no doubt that in the future a greater percentage of positive cultures will be isolated from specimens of blood from suspected cases of undulant fever. More laboratories are becoming familiar with suitable methods of growing Br. abortus and Br. melitensis. Until comparatively recently only a few laboratories engaged in the study of infectious abortion were familiar with the peculiar cultural characteristics of the organisms in this group. In our series of the first fifty cases studied, we succeeded in obtaining cultures from only one-third of the patients. We are now able to grow cultures of Br. abortus from about 60 per cent of the samples submitted for bacteriologic examination. Kristensen reports that he has succeeded in recovering cultures from 65 per cent of his patients.

Because of the varied symptomatology of undulant fever and the limitations of the agglutination test, and because Br. abortus is not isolated from cases of this disease, it is very important that both the bacteriologist and the clinician have as much information concerning the patient as possible before a diagnosis is made.

Although great care should be exercised in handling cultures of Br. abortus and Br. melitensis, we believe that technicians and bacteriologists should not be unduly alarmed concerning the danger of working with these species. They cannot be so dangerous to work with as Bacillus anthracis, Pfeifferella mallei and the virus of rabies. As everyone knows, unnecessary fear causes more catastrophies than intelligent care in handling such infectious agents.

It appears that we have only made a beginning in the study of this disease. The bacteriologist must possess patience and perseverance, as well as a thorough knowledge of the cultural characteristics of Br. abortus and of its serologic peculiarities to be of value to the practitioner in establishing a correct diagnosis of undulant fever.

NOTE: We are very much indebted to the Metropolitan Life Insurance Company for their financial support of our studies on undulant fever.

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SOME OBSERVATIONS ON THE AGGLUTINATION OF BR. ABORTUS*

By Frank B. Lynch, M.D., and Annette M. Callan, B.A., Philadelphia, Pa.

DURING a period of eighteen months, seven agglutinations for Br. melitensis, var. abortus, have been obtained in studies made in this laboratory out of 100 agglutination tests for that organism. Two were on patients admitted to the hospital, one a clear-cut medical case and the other admitted for tonsillectomy, following which, due to continued fever, this infection was discovered. The other agglutinations were done on sera submitted to the laboratory.

Both cases were considered as possible typhoid infections, but after negative findings the sera tested were agglutinated by Br. abortus, the adult medical case in a dilution of 1:1280 and the child of fifteen years in a dilution of 1:640.

From this experience developed a desire to prepare a safe, fairly stable and readily agglutinable antigen to be used parallel with the Dreyer method for typhoid agglutination. Our first method has continued with practically no change, and it is possible that our experience may be profitable to others.

Three strains of Br. abortus were obtained from the Laboratories of the State Live Stock Board, and a bacterial suspension of each was used against a known positive serum. The one giving the highest titer and clearest cut reaction was selected for stock culture. It is carried on 3 per cent glycerine, 1 per cent glucose agar.

The organism for antigen is incubated seevnty-two hours on slants in large tubes (approximately 15 by 2 cm.) on 3 per cent glycerine, 1 per cent glucose agar. Six to 8 slants will yield about 200 c.c. of antigen. The growth is then emulsified in 0.85 per cent NaCl and killed by heating in a water-bath at 55° C. for forty-five minutes. It is then controlled for sterility and filtered through sterile cotton; shaken in a vaccine bottle with beads, and diluted with 0.85 per cent salt solution to an opacity equal to about 3 billion staphylococci per c.c. We store this antigen in a refrigerator, and have used no preservative, but 0.5 per cent phenol could probably be added without loss of antigenic value.

The test is set up in tubes 75 by 14 mm. in size, providing four rows of 10 tubes each as for a Dreyer Widal, the last row being for Br. abortus. The patient's serum is set up in all rows in dilutions of 1:10, 1:20, 1:40, etc., and to the first row 0.75 c.c. of B. typhosus antigen is added, to the second, Para A, to the third, Para B, and to the fourth Br. abortus antigen. Place in a water-bath at between 50°-55° C. (best about 52° C.) for two hours.

As a check on this test, 10 control tubes are included, using a negative serum plus saline, a positive serum plus saline, a positive or immune serum,

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From the William Pepper Laboratory of Clinical Medicine, University of Pennsylvania.

saline and respective antigen for each row, and the same with a negative serum. After two hours remove rack from the water-bath, take preliminary reading and place in the ice box for six to eight hours, or better, overnight. We have found this has given clearer cut reactions or has sometimes raised the titer one or even two dilutions.

The reactions are read on the usually accepted basis of 4+ for complete flocculation and clearing; 3+ for 75 per cent clearing; 2+ for 50 per cent clearing; 1+ for partial clearing and 0 for no reaction.

Although our experience has been limited in the discovery of so few positive infections, we have considered much of the literature on this infection and have used other recommended methods for this agglutination. Our method seems to have given reliable results on a minimum of added procedure, with no special equipment, no separate test, no seemingly difficult standardization of an antigen.

In comparison with other methods, we have made the following observations on all sera submitted during eighteen months on which Widal and Br. abortus tests have been performed:

1. Antigen, prepared as indicated, has given clear-cut reactions at least two months after its preparation, and tests done on ice box stored antigen over five months old have given 90 per cent clearing on the same sera. Titer on two months' old antigen was unchanged. Handled under sterile precautions, no contamination has occurred.

2. In a study of system controls, the following observations have been made on positive sera:

a. In all typhoid and paratyphoid agglutinations, the reaction was definite or almost complete in the first fifteen minutes of incubation.

b. In Br. abortus strain, agglutination did not begin earlier than one hour after incubation had begun, and continued slowly during the next hour. It was complete in two hours.

3. Relative character of agglutination:

a. In all typhoid strains, the bacterial agglutination was light, feathery, and on agitation returned to almost complete bacterial suspension.

b. With Br. abortus, the bacterial agglutination was coarsely granular, and on agitation did not return to suspension. It settled again almost immediately. We think this an important and useful observation.

4. Time of incubation. It is believed that this period is sufficient for agglutinations within the range of any dilution accepted as of clinical significance. We have never obtained an agglutination in a dilution of less than 1:20. We have reported none on a dilution less than 1:50 and then with reservation.

5. Inactivated sera. We have not inactivated our sera on routine tests. Studies were made on 25 sera, both inactivated and uninactivated, and this method has shown no difference in results. All were negative down to 1:20 dilution.

Reference to the literature shows a variation in the recorded thermal death point of Br. abortus from fifteen minutes at 60° C. to two hours at

60° C. These results in many cases refer to suspensions in milk. We have found that our heavy suspensions in salt solution have invariably been killed by heating for forty-five minutes at 55° C., and feel there is probably less alteration of the bacterial structure at that point than at higher temperatures.

The incidence of human infection with Br. abortus is variously indicated in different reports. This is probably not due entirely to local variations in the prevalence of the disease, but partly to the various groups from which the material is drawn.

McAlpine and Wedeman[1] report a 0.6 per cent incidence of human infection with Br. abortus in a state in which 90 per cent of the cattle were found to be infected and only 60 per cent of the milk pasteurized. This figure represents the positives obtained in a study of over 10,000 human sera submitted for Wassermann test.

Our series, on the other hand, represents about 7 per cent positive agglutinations for Br. abortus, in a series of 100 cases, most of whom had febrile symptoms, and many of whom were at first thought to be typhoid fever. It is obvious that the incidence would be higher in such a group.

In the inactivation of sera, Négre and Raynaud[2] cite instances of false positives on unheated sera, which are avoided if sera be heated to 56° C. Evans[3] reports in her study that inactivation did not reduce percentage of positives and Hardy recommends inactivation at 56° C. for thirty minutes, if a 37° C. water-bath be used for the test.

We might remark here that although we have not inactivated our sera, we feel that 50 to 55° C. for two hours has inactivated our sera within the first twenty minutes. If bacteriolysis occur during this time it is probably slight.

We have used in our positive control study an immune serum now a year old. This serum certainly contains no complement.

Rapid macroscopic agglutination. The method of Huddleson[4] has been studied on a limited number of cases. It has not discovered any positives other than those we found by our method, which in addition gives a titer on the case. This method is doubtless useful where many sera are to be examined. The higher percentage salt solution (12 per cent) renders the antigen likely to crystallization, a disadvantage where occasional tests are made. We did not get clear-cut reactions except in cases of positives of high value.

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NOTES ON THE BACTERIOLOGY OF THE BRUCELLA GROUP*

By K. F. Meyer and B. Eddie, San Francisco, Calif.

IN THE study of infectious diseases, it is the aim of the pathologist to recognize the significance of finer distinction between strains of pathogenic organisms, the purpose and the limitations of the response to infection by the host, and the process of stimulation and adjustment of immunity reaction to meet the peculiar conditions of the case. These fundamental prerequisites must be applied to the study of the problem of undulant fever and the organisms which cause this disease. Only too frequently, the early inquiries are inspired by the needs of diagnosis and the wish to prevent or to cure, and relatively little is done in the analysis and the interpretation of the process and progress of the infection. When the authors resumed, a few years ago, the study of undulant fever, it was realized that much could be gained if the inquiries would be disinterested and would primarily deal with an investigation of the tolerance and antagonism that the specific organism meets in animals which it infects. Although primarily interested in this phase, it became necessary to compare the cultures which were used in the study. Since previous papers from the Hooper Foundation had shown that the main representatives of the Brucella group, Br. abortus and Br. melitensis, cannot be distinguished by morphologic or biochemical criteria, the agglutinin absorption test was chosen as the method of differentiation. First employed by Feusier and Meyer[1] and then developed by A. Evans[2] on a broad basis, this procedure led to the recognition of at least eight serologic groups. Every strain used in the experiments on animals has been compared with at least two authentic strains representing the main varieties observed in the United States. Contrary to previous experiences, the final identification was very difficult since a number of new serologic nuances and varieties could not be correlated with the standard types. Particularly, the strains from European countries have shown many intermediate types which still await final serologic identification. Some are new melitensis types, others are probably subvarieties of the para-abortus group. Until the time-consuming studies have been completed, it is impossible to decide the pertinent question: Is the serologic classification a superficial and perhaps a valueless complication which does not coincide with the invasive power and the pathogenic behavior? The author is prepared to admit that he has overestimated the significance of the agglutinin absorption test. It is not unlikely that the Brucella group of organisms is derived from a common stock which has differentiated and is still differentiating in their relationship to man and animals. In the environment in which the author has made most of his investigation, the abortus

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variety is responsible for the placental disease of cattle, but Evans,[3] Huddleson,[4] and others have proved directly or indirectly the occurrence of true melitensis varieties in bovines. Therefore, the general assumption that the true melitensis is primarily a parasite of the goat and the patients with melitensis septicemias have been infected through caprine sources may need revision. Thus it must further be concluded that the serologic identification, if successful with the authentic strains at hand, does not in all probability determine the source of the variety. In other words, the isolation of a melitensis from the blood of a patient is no proof that he or she contracted the disease through goat's milk or goat's milk products. Furthermore, the demonstration of a melitensis variety in cow's milk does not establish the goat as the source of the bovine infection. In fact, it is as yet unknown where and how these bovine-melitensis strains first originated. It would be interesting to study the serologic behavior of a Br. melitensis after prolonged sojourn in an aberrant host like the cow. Burnet[5] has investigated the effect of the abortus on goats, and although he found the organism to be less adapted to this ruminant, he has not considered the possibility of an adaptation through passage.

The abortus varieties have remained cosmopolitan in their activities; they infest aside from cattle, hogs, sheep, and horses, and in place of acquiring a more specific virulence, they have widened the range of their less specialized attack, and developed a more potent toxin. This is indicated by the pathogenic behavior of the abortus strains of porcine origin which has been carefully studied by Th. Smith[6] and amply confirmed by other workers. The selection of the guinea pig to differentiate the porcine from the bovine strains has added a new method to the procedures which may be used in the classification of the Brucella organism of human origin. These pathogenicity tests established Br. abortus of porcine origin as the causative organism of human undulant fever. But since a number of workers have found this variety which cannot be separated from the true bovine abortus by agglutinin absorption tests in cow's milk, the old question, "where did the human patient contract his infection?" remains undecided. Furthermore, the pathogenicity of the abortus strains of porcine origin may be low and resemble that of the bovine varieties (Smith[7]). Under these circumstances, differentiation by animal tests may be difficult. In this connection, it is recalled that the pathogenicity of a human Brucella organism recently isolated from a case of undulant fever in the southwestern part of the United States may be feeble or nil for guinea pigs or a strain, serologically a true melitensis, may a few days after its isolation produce typical bovine lesions. These observations, kindly confirmed by Dr. Th. Smith impose certain limitations on the pathogenicity tests for differential purposes. Equally unsatisfactory have been the thermoagglutination tests as advocated by Ficai and Alessandrini,[8] the differential allergic reactions (Fleischner and Meyer,[9] Polettini,[10]), the cross immunization experiments of Zibordi,[11] the nonspecific acid agglutination test with different H-ion concentrations of lactic acid according to Vercellana and Zanzucchi[12] (see also Beguet,[13] La Rosa,[14] Cerruti,[15] Favilli[16] and Andrei[17]), and the thermolipoidal precipitation reaction of Valenti.[18] Personal experiences with the

thermoagglutination and the Valenti test justify the conclusion that they are both valueless.

At this stage of the investigations, studies on the nutritive substances, growth accessory factors required by the Brucella organisms, the effect of salts, the nature of the enzymes, etc., have been undertaken. The work will be reported when completed. To date it has not furnished any definite proof that biologic means may be found which may readily distinguish the varieties of the Brucella genus. The experiments supply, however, important information regarding the virulence and toxin production in relation to the culture medium. While working on these phases of the problem, two new methods have been proposed for the differentiation of the varieties of the genus Brucella. One developed by McAlpine and Slanetz[19] makes use of the differences in the glucose and nitrogen metabolism which can be tested in broth cultures of the Brucella organism and the other, recommended by Huddleson,[20] determines the growth differences of the species by use of dye media. According to the first named workers, certain striking differences in the utilization of glucose by the varieties of the Brucella group show that the abortus-melitensis group may be subdivided into two main divisions. The first, known as the abortus group, comprises strains which are unable to utilize more than 2 per cent glucose in the presence of Fairchild's peptone and consequently demize the amino acids and render the medium increasingly alkaline. The second group includes the porcine and melitensis strains which hydrolyze from 5 to 20 per cent of the carbohydrate and during the first six days of growth increasing acid values over the control figures may be recorded.

Later, McAlpine and Slanetz[21] found that these differences in the metabolism are closely paralleled by the behavior of the strains toward carbon dioxide. Thus the growth of the abortus cultures is accelerated by the cultivation in an environment containing 5 to 10 per cent CO_2, while the porcine and melitensis cultures are more or less inhibited when incubated under the same conditions. In brief, distinct metabolic differences exist between the porcine and bovine varieties. However, these procedures fail to differentiate between the porcine and melitensis varieties. Huddlson[4] has supplemented these shortcomings first by a study of the rate of H_2S production and then by a comparison of the genesistatic properties of certain dyes to interfere with the reproductive mechanism of the representatives of the genus Brucella. According to the time and rate of H_2S production, genesistasis toward thionin, methyl-violet and basic fuchsin he subdivides the Brucella into three species: Br. abortus, Br. suis, and Br. melitensis. Whether or not this classification is justified remains to be determined. The designation, Br. suis, is probably premature since recent observations indicate that swine may become infected with the true abortus indistinguishable from the bovine variety. Tentatively the author has adopted the terminology of Huddleson to record concisely the selective genesistatic potency of certain dyes on the representatives of the Brucella group: Bovis, thionin genesistatic, fuchsin and methyl-violet fast. Suis, thionin fast, but fuchsin and methyl-violet genesistatic. Melitensis, thionin, fuchsin and as a rule, methyl-violet fast. But since the mechanism of the selective bacteriostasis is unknown, it is believed that this interesting method

should not be used indiscriminately in the determination of the possible source of undulant fever in man.

During the past year the procedures of McAlpine and Slanetz and those of Huddleson and his associates have been used in the Hooper Laboratories and are the subject of biochemical analysis. The glucose utilization tests have been supplemented by a determination of the nature of the fixed and volatile acids which are formed by the various Brucellas. The data have been collected by a worker who was not acquainted with the histories or origin of the cultures. Recently some of his findings have been correlated with the serologic data and the results on the dye plates. The duplication of the differential test devised by Huddleson at first offered considerable difficulties since Grübler pre-war and Coleman and Bell dyes were used. After being advised by Dr. I. F. Huddleson that he works entirely with Certified National aniline dyes, the behavior of certain authentic strains was found to correspond more closely to those published by him. Since the observations with different dyes will be the subject of a separate publication from this laboratory, it is unnecessary to enter into a discussion of the discrepancies. Suffice it to indicate that for example the Grübler thionin was rarely inhibitive for the "abortus" while the Coleman and Bell dye even exerted a bacteriostatic effect on the "suis" strains. In all probability the presence or absence of impurities may control the selective genesistatic effect of certain dyes. It is the purpose of the biochemical studies which are now in progress to determine the nature of these substances. Until the value of this differential method for the Brucella group has been determined by workers throughout the world, it is important to specify the use of National aniline and not merely certified dyes. If this prerequisite is not considered results as those recently published by Saitta[22] from the laboratory of Professor Polettini in Cagliari in Italy will rapidly discredit a test which deserves further study.

Over 130 cultures of Brucella have been tested at least three and some six times on media containing different dilutions of the dyes. Certain strains have shown variable sensitiveness toward thionin. Recently isolated organisms classified serologically as melitensis may be inhibited by gentian-violet in a dilution of 1:50,000 and 1:100,000 and give faint growth in 1:250,000. As a rule, the behavior toward basic fuchsin has been rather constant. The strains either grow in every test or they are regularly inhibited. However, reduction of the dye may accompany the growth of certain abortus and few melitensis strains. The significance of this phenomenon is unknown.

Some of the observations worthy of note are briefly as follows: Twenty cultures from bovine and one from porcine sources in Switzerland may be classed as "bovis." Of eight cultures obtained from Germany as true "abortus" strains, three behave like "melitensis" and five like bovis types; of four Hungarian cultures, one behaves like a melitensis while three Italian cultures are identified as "bovis" varieties. Equally interesting are the findings on the human cultures. In general, the strains classified serologically as "melitensis" reacted as such on the dye media; those identified by agglutinin absorption tests as abortus were either linked with the "suis" or the "bovis" types. With one exception, twenty cultures sent by Dr. A. V. Hardy from

Iowa are "suis" varieties; some of the New York strains fall into the same group while the Michigan cultures are mostly "bovis" types. Twelve strains isolated by Dr. M. Kristensen in Denmark and one human strain obtained from Dr. H. Habs and isolated at Kiel, Germany, react like "bovis" and four recent cultures from Tunis like "melitensis" types. However, one recently isolated California strain and an old laboratory culture (426, our No. 20 Austria) serologically abortus varieties, reacted like "melitensis." According to Huddleson, an equine strain studied by A. Evans as a melitensis possesses also a dye sensitiveness like a "bovis" type. Through the courtesy of Dr. Huddleson, the behavior of the irregular German, Hungarian, Austrian, and Californian strains toward dyes has been confirmed. Strain 6454 from a bovine placenta utilized 3 to 5.4 per cent glucose in seven days, is inhibited by 20 per cent CO_2 and is serologically an abortus. The two other German and one Hungarian abortus obtained from fetuses gave repeatedly low glucose utilization figures and grew well at 20 per cent CO_2. The California strain (Love) hydrolyzes 4.2 per cent glucose, grows freely in CO_2 and produces a progressive amount of H_2S in liver and in cystin veal infusion agar. It produces "bovine" lesions in guinea pigs but infects monkeys like a "melitensis."

As a whole, the study of the glucose metabolism has not furnished the differences one would expect from the publications of McAlpine and Slanetz. Quite a number of abortus cultures utilize more than 2 per cent while several "melitensis" strains split the carbohydrate in amounts below 5 per cent. On the other hand, it is evident that the feeble biochemical activity of the majority of the abortus strains may be contrasted by the vigorous carbohydrate utilization of certain porcine and the Tunisian melitensis cultures. The two main groups are, however, joined by strains which from a differential diagnostic standpoint, consume an indecisive amount of glucose. It may be mere coincidence but the biochemically aberrant strains reveal also a sensitiveness to dyes which cannot be correlated with the serologic reactions. What is the meaning of these biologic nuances? Are they new species in the course of formation or are they new host adaptations? The limited data hardly justify conclusions. However, it is evident that the previously expressed view concerning the plasticity and variability within the Brucella group is again expressed in the biochemical and dye sensitiveness tests. Until their origin is known, their importance in the epidemiology of undulant fever belongs to the realm of speculation. Even if it is assumed that Brucella organisms which according to the differential tests, belong in the "melitensis" group may infect cattle and consequently man, it still remains to be determined why, for example, in the observation of Carpenter and King[23] only 6 of 150 or more persons who partook of the milk became infected, or why in the case of Frei[24] the milk of an abortus infected goat was ingested with impunity by a volunteer. In all probability the problem is not so much the discovery and classification of the varieties but the recognition of the tolerance and antagonism that the Brucella meets in the animals or human beings it infects. The low human population susceptibility which according to Orr and Huddleson[25] is only 1.4 per cent, when exposed constantly to the abortus organisms has, as far as is known, not received further investigation. If only those individuals

who develop agglutinins or clinical manifestations of the disease are to be considered infected, then it must be held that the vast majority of persons in northern countries in which the placental disease of cattle is common, possess a tremendous natural immunity against the Br. abortus and its varieties. From a clinical standpoint, this view may appear justifiable but bacteriologically other possibilities exist. Either the constant ingestion of a small number of Brucella organisms in cow's milk during infancy may lead to an acquired immunity or a carrier state. In this connection the discovery of the abortus bacillus in the tonsils of children by Mohler and Traum[26] in 1911 deserves further investigation. From this point of view probably few people exhibit any natural immunity and whether they develop signs and symptoms of the disease depends upon the intensity of their exposure to the infection and the reaction of the host. The nature of the main defense and the mechanism leading to the immunity are not known, although Ninni[27] in Rome has recently demonstrated a strong bactericidal action of normal human sera on the Bang's but not on the majority of the Bruce melitensis bacteria. It is these phases of the undulant fever problem which have interested the workers in the Hooper Foundation. Preliminary to the studies on man the susceptibility of monkeys to the representatives of the Brucella group has been investigated on 88 monkeys. The following observations have been made:

A single oral administration of 21 different Br. abortus strains produced in 24 Macacus rhesus and 1 M. cynomolgus monkey nonfebrile infections, followed by the formation of specific agglutinins of moderately high value. The dosage varied from 7 to 400 millions and in some experiments it consisted of many billions. The course of the clinical disease is not markedly influenced by the number of bacteria which are fed. Two cynomolgus and one rhesus monkeys were refractory. The strains identified serologically as abortus or para-abortus varieties and in the dye test as "bovis" or "melitensis" types had been isolated from bovine pathologic specimens in the United States, Germany, Hungary, Italy, and Switzerland. Blood cultures have not been successful. The value of the serum agglutinins and their persistence depends on the feeding dose. Rapid disappearance of the agglutinative power to a low titer or to the zero point is worthy of note. A cutaneous application of approximately 20,000 bacteria has induced an infection. The incubation period as indicated by the appearance of the serum reaction varied from nine to thirty days and is influenced by the infective dose. In general, the smaller the dose the longer the incubation time. The absolute evidence of infection has been secured through the recovery of the organisms from the tissues of four monkeys which have been sacrificed on the thirty-fourth to fifty-second day. Three animals killed on the forty-third, fifty-sixth and one hundred ninety-ninth day furnished sterile cultures. Probably every Br. abortus strain when fed in sufficiently large dosage is pathogenic provided susceptible monkeys are used. It is conclusively proved that even old laboratory strains may penetrate the intestinal mucosa and stimulate the production of antibodies. In general, the validity of previous conclusions that the Br. abortus is less pathogenic than Br. melitensis is confirmed, but the differences are less marked

probably on account of the greater variety of cultures, and the larger series of animals which has been studied.

By feeding 100 million Br. abortus type "suis" of bovine but in all probability of porcine origin which has retained its characteristics through the passage, a febrile disease with anatomic lesions indistinguishable from those of a Br. melitensis infection of the monkey has been produced. Repeated feedings of 35,000 bacteria, cutaneous application to the shaved skin and intravenous injection of the organism has given the same result. Although the blood stream sterilized itself rapidly, the causative Brucella organism is readily isolated in enormous numbers from the hemopoietic organs and lymph nodes provided the monkeys are sacrificed within thirty to fifty days after the administration of the infective material. The incubation time dependent on the dose varied from six to twenty-one days. The maximum value of the agglutinative power of the serum in part controlled by the feeding dose persists for several weeks, and a somewhat lower titer for many months. However, the persistence is not any longer than that observed in monkeys infected with Br. abortus or Br. melitensis. During artificial cultivation the febrigenic properties on feeding have been lost but they have been retained by one strain when the organisms are applied cutaneously. The milk of the cow which furnished one of the pathogenic suis strains has been consumed by a group of people without any bad effects.

An old laboratory culture of a Br. abortus type "suis" of porcine origin infected via the alimentary tract when fed in large doses. The infection ran an afebrile course, stimulated after an incubation time of from nine to ten days, a powerful agglutinative value of the serum with an abundance of specific organisms in the tissues.

A Br. abortus type "bovis" isolated from a swine fetus infected and immunized a monkey in a manner similar to that of the "bovis" types of bovine origin.

Melitensis strains of American origin possess a low virulence for monkeys; they may act like "bovis" cultures, and they may lose their pathogenicity entirely within six months of artificial cultivation. Test tube strains several years old are nonpathogenic and when administered by mouth they lack immunizing properties. One culture, which produced no lesions in guinea pigs by injection, infected a rhesus by mouth.

Tunisian strains of Br. melitensis fed or inoculated in doses of 100 million bacteria give rise to a febrile disease which is generally considered characteristic for this group of organisms. Even recently isolated strains may induce merely serologic but no febrile reactions.

A Brucella organism serologically and biochemically an abortus and in its behavior toward dyes a melitensis type, acted like a typical melitensis by feeding and inoculation one month after isolation from a California patient with undulant fever. In contrast, nine other strains kept under artificial cultivation for from one to twenty-four months and isolated from human abortus fever cases in Michigan, Iowa, Northern Germany and Denmark, infect monkeys when fed in a manner characteristic for the Br. abortus "bovis"

type. Febrile reactions may be recorded on intravenous inoculation of large doses (one rhesus). No specific thermic response has been noted following the separate subcutaneous injection of two Danish human abortus strains.

The agglutinative power of the serum with few exceptions remains low and is frequently of short duration even following the administration of massive doses via the alimentary tract.

An old type culture No. 20 (426, A. Evans) originally from Austria, isolated prior to 1915, serologically an abortus and on dye media a melitensis type, readily penetrated the intestinal mucosa and immunized against a virulent infection with a "suis" type. Three Br. abortus type suis strains of human origin have not exhibited any striking pathogenicity or marked febrigenic properties, neither by feeding nor by cutaneous or intravenous injection. They behave like test tube strains of the melitensis variety and show evidence of rapid deterioration under artificial cultivation. A fourth culture with a history which suggests a laboratory infection with virulent Brucella organisms of porcine origin produced a typical Brucella septicemia with a marked pyrexia and a rapid serologic reaction following the oral introduction of 182 million bacteria.

Serum agglutinins specific for the Brucella group are formed only in the presence of a definite infection. The ingestion of heat-killed abortus bacilli with or without bile is antigenically ineffective in monkeys and rabbits.

Over 10 per cent of the rhesus and cynomolgus monkeys possess a natural immunity against Brucella infections via the alimentary tract. Animals which react to the oral administration of virulent abortus organisms with moderate and in general transitory serum reactions resist subsequent feeding infections with Br. abortus "bovis" and "suis" but not with a Tunisian Br. melitensis. The inherent and the acquired local intestinal immunity may be broken by an intravenous or subcutaneous injection of a virulent type "suis" or "melitensis" strain. An alimentary introduction of living and moderately virulent Brucella organisms and their varieties may readily vaccinate rhesus and cynomolgus monkeys. The protection thus afforded is relative and its mechanism deserves further investigation since it may explain certain puzzling features of the epidemiology of abortus undulant fever in the countries in which abortion disease in cattle is prevalent. There are indications that previous contact with noninfective doses of Brucella organisms may render the animal hypergic to massive parenteral infections. Continuous ingestion of small numbers of abortus may lead to mild, unrecognized or "silent" yet immunizing infections. At least in one observation, the local and general immunity thus induced has been definite.

Final conclusions concerning the relationship of the different Brucella varieties to human undulant fever in this country cannot now be drawn. The evidence incriminates Br. melitensis var. abortus of bovine origin as the causative factor in a fairly large percentage of carefully studied cases. But why and how these cases occur remains to be determined. The bacteriologist who must assist the epidemiologist should appreciate that the cultures should be tested by the differential methods as soon as possible after their isolation. In particular pathogenicity tests on monkeys and guinea pigs should not be

delayed. The behavior of these strains following bovine, caprine and porcine passage should be scrutinized and the process of stimulation and adjustment of the immunity reactions which the animal body may mobilize in order to ward off Brucella infection, deserves detailed consideration. The pathologist's contribution to the problem of undulant fever lies in the careful analysis and interpretation of the process and progress of the infection in combination with the biologic analysis of the causative organism.

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DISCUSSION OF PAPERS BY GIORDANO AND SENSENICH, CARPENTER AND BOAK, LYNCH AND CALLAN, AND MEYER AND EDDIE

Dr. Walter M. Simpson.—I was surprised to find my name on the program because I have no formal paper to present. I have prepared a paper on undulant fever for the Section on Pathology and Physiology of the American Medical Association, to be read next week. However, to open the discussion I will tell you some of our experiences with undulant fever. During the past year we have carried out a clinical and pathologic investigation of sixty-three cases of undulant fever in Dayton. The experience of Evans, Carpenter, Huddleson, Hardy and ourselves would indicate that if an individual undertakes a survey of undulant fever in any given place, he will be surprised at the large number of cases which will be discovered. It is generally known that infectious abortion of cattle and domestic animals is widely prevalent. Our own experience would indicate that the disease is very common in southern Ohio. Of sixty-three cows in four herds that supplied milk to ten of the Dayton patients, all but four calves gave serologic evidence of Brucella abortus infection. Five of these cows were eliminating the organisms in their milk in large numbers. Cultures derived from the blood of one human case and from the milk of three of the cows were submitted to Huddleson, who found that they were all of the bovine type.

As to the clinical manifestations of the disease they have been completely described by Dr. Giordano. It has been repeatedly stated that there is no well defined clinical picture of this disease. Evans has stated that there is no other disease in which the clinician is so dependent on the laboratorian. Our experience has not been in accord with this widely-accepted belief. An initial clinical diagnosis of undulant fever has been made by several Dayton physicians and they have asked us only to confirm their clinical diagnoses. As regards the sexual incidence, we found both sexes to be about equally affected. In six instances the disease occurred during the first decade of life; the youngest was a child of six. The greatest incidence occurs between 20 and 40. Twenty-two of our patients were housewives; only 9 were farmers or dairymen; seven were university students, and the remainder were engaged in nonagricultural pursuits. More than one member of six families was affected. Seven of the patients had been admitted to the hospital with a diagnosis of acute rheumatic fever; all showed acute joint manifestations. Abdominal pain was a prominent feature of the disease in eleven patients; in five of these there was lower right quadrant pain with fever; appendectomy was performed in three cases. In one of these an acute gangrenous appendix was found. The other two appendices were entirely normal and cultures were negative. In one case of upper right quadrant pain, accompanied by chills, fever and sweats, cholecystectomy was contemplated.

In view of the fact that Brucella abortus derives its name from its known tendency to cause abortion in animals, we wondered whether or not it might be a factor in certain cases of human abortion. We found five women who had repeatedly aborted, whose serum agglutinated Brucella abortus in titers from 1:80 to 1:320; four of the five gave a history of a previous febrile illness, the exact nature of which was not established at the time. All were raw milk consumers.

It is believed by many that the disease may be transmitted to cows by the bull through the seminal route. Three of our male patients went first to genitourinary surgeons because of orchitis, epididymitis and prostatitis. There was no history of gonococcal infec-

tion and repeated examinations failed to reveal any gram-negative diplococci. The sera of these men agglutinated Brucella abortus in titers of 1:160 to 1:640. In one of the cases we recovered Brucella abortus from a sinus tract extending through the scrotal wall.

In three of our patients there was an enlarged spleen and an eruption simulating the roseola of typhoid fever. Six of our patients were confined in a local tuberculosis sanatorium; all had been admitted with a diagnosis of tuberculosis.

There are a few cases in which antiabortus agglutinins do not develop. There appears to be need of a more delicate test to supplement the agglutination reaction. The intradermal test seems to hold considerable promise. In ten cases, with positive agglutination in titers from 1:160 to 1:1280, we carried out skin tests using 0.1 c.c. of a suspension of heat-killed organisms adjusted to the turbidity standard used in the preparation of antigen for the agglutination test. In each instance a strongly positive local reaction occurred. On the same patients, we later carried out a skin test with antigen sent to us by Giordano with similar results.

For practical purposes we adopted the standard of regarding all cases in which agglutinations occurred in titers of 1:80 or above, or in which we recovered the organism, as evidence of past or present infection with Brucella abortus. If an individual is sick and his serum agglutinates Brucella abortus, it does not necessarily mean that that individual is suffering from undulant fever at the time of the test; the agglutination may be the result of a previous experience with undulant fever, months or years previously. In those cases in which the agglutination titer is 1:10, 1:20 or 1:40, further bacteriological and serological studies should be carried out.

As to the treatment, we have used in 29 cases a vaccine which was prepared from killed abortus organisms, standardized to 2 billion per c.c. Graduated doses were given, beginning with one-fourth c.c. at two- or three-day intervals. Following the first injections, there was usually a transient rise in temperature. With three exceptions, the fever rapidly returned to the normal level after two to six injections. Injections of milk or typhoid vaccine did not give equally good results. I am no vaccine enthusiast, but I do believe that this matter is worthy of further study by other workers. It is a very difficult thing to estimate the value of any vaccine therapy in a disease which is characterized by spontaneous remissions.

Dr. Frank W. Hartman.—I would like to ask if any of the essayists have seen this in children. We had a case in Detroit a year ago in a child eight years old. The parents insisted it had never had anything but certified milk. They made things very unpleasant for the dairies in the city of Detroit. That child responded to multiple transfusions.

Dr. C. W. Maynard.—I simply want to ask a question and hope to be answered from the standpoint of the public health laboratory. We have about half of our milk supply pasteurized and the other half from raw milk dairies. For two years we have looked for undulant fever, not in as concentrated a way as some of the men have been doing, but we have been looking for it and have been using antigen from the hygienic laboratory. We had one case that has been proved. Should we public health officers insist upon the other half of our milk being pasteurized? Many prefer raw milk. When we have these cases how long should we keep them under public health supervision? Should we be lenient with them or keep them under supervision as long as we can isolate the organism from the urine?

Dr. R. L. Sensenich.—It has been well pointed out in this symposium that the clinical course of the disease may make the diagnosis possible or the absence of characteristic evidence may throw the diagnosis into the realm of the clinical pathologist. It is our experience as Dr. Simpson has reported in his series of cases that the diagnosis in a great many instances may be made clinically provided an educational campaign is carried on among the physicians of the community as I understand Dr. Simpson has done. The illness may be very short and the character of the infection unsuspected. Dr. Lynch's presentation is interesting as I believe that some of these cases of short clinical course ultimately appear among those in whom the blood findings may be positive and yet there may be no other evidence of undulant fever. The cases of more protracted illness usually display clinical phenomena which are sufficiently characteristic to make the diagnosis comparable to that of typhoid fever in which clinical conclusions are confirmed by laboratory tests. In

the acute cases the situation is different. However, any acute septic type of illness with chills and fever, without readily demonstrable infection, should suggest undulant fever and indicate the advisability of blood studies. The presentation of the paper of Dr. Giordano's and mine was an effort to further describe and classify the clinical types of the disease encountered and point out the frequency of a relatively short, acute course in the belief that the diagnosis will be more often made if physicians have the possibility of undulant fever in mind. Specific treatment has been unsatisfactory. Nothing has taken the place of bed rest. Fortunately, a surprisingly large number of cases run a very limited course and make a complete recovery.

Dr. A. V. Hardy.—In our study of undulant fever in Iowa we have been impressed with the fact that time after time we would come to farms and find the man infected and the woman not involved. The proportion of infected men to women living on farms is 9 to 1. Other investigators in the United States have had similar findings. This observation led us to believe that the skin might be the portal of entry. We undertook an experimental investigation of this point. We used guinea pigs and we selected two organisms, one a porcine strain which gave very marked pathologic lesions, and the other the bovine type. We prepared the organisms by growing on agar for forty-eight hours. We washed these off and diluted to compare with the 500 p.p.m. opacity standard. We used four different methods of exposure. First, shaving the skin and abrasing. Second, carefully shaving the skin, no apparent abrasion. Third, clipping the hair. Organisms (0.2 c.c.) were applied and spread over the prepared area with a glass rod. The fourth group we fed the same amount by mouth. We were quite surprised with the results. Of the pigs in which the skin was shaved and abrased, 100 per cent of the pigs were infected; those shaved with no abrasion, 90 per cent were infected; those with the hair clipped, 78 per cent, but in those fed by mouth only 22 per cent. Four times the number were infected by contact with the skin than with feeding. Dosage has a great deal to do in determining whether infection will be acquired and further experiments confirmed this belief.

We were particularly fortunate also in being allowed to study a group of packing house employees. We had diagnosed seven cases in that packing house and the men in charge agreed to ask the men to report for blood examinations. We found of the 217 bloods obtained, 29 or 14 per cent reacted in the titer of 1:80 or higher. The proportion infected varied depending on the intimacy of contact with fresh tissues. Less than half of those giving laboratory evidence of infection gave any history of undulant fever and only three had had clinical diagnoses.

I feel that the portal of entry must not be considered a closed question. It seems clear that there is more than one portal of entry, and we should study to determine the relative importance of the different sites of entrance of infecting organisms. The skin as a portal of entry must be given more consideration.

Dr. C. W. Bonynge.—Regarding joint involvement in undulant fever—I think we have had twelve to fifteen cases of undulant fever in Los Angeles, and in speaking with Dr. Hammack, we both remarked that none of these have shown joint involvement. I am glad this point has been brought out by the speaker.

Dr. Maynard has also brought out an interesting point, the occurrence of undulant fever in infants. We have been led to believe that infants are immune. We had one case in an eleven months old child who had been on certified milk for nine months. It would be hard to question that this was a milk-borne infection. However three other children in the same family, using larger quantities of the same milk, were not infected.

It would seem that we still have much to learn regarding undulant fever, especially of the etiology.

Recently the Los Angeles Certified Milk Commission has ruled that our certified dairies must rid themselves of infected animals as determined by the agglutination test. The herds are repeatedly retested and only negative animals may be added. Since May, 1929, no certified milk has been served except from negative cows.

The certified dairies of the San Francisco district have been abortus free for nearly fourteen months and continuous examination of the milk has given negative results for Brucella abortus.

Dr. Charles M. Carpenter.—I have isolated Brucella abortus from seven of fifty-five pairs of tonsils. We have found the tonsils and lymph nodes of calves to become infected first and to remain infected the longest of any of their tissues. I want to mention a marked difference in the results obtained by Huddleson from those reported by McAlpine and Slanetz. The former stated that according to the inhibitory effect of dyes on Brucella melitensis it is similar to Brucella abortus of bovine origin while the latter, who studied the utilization of glucose by Brucella melitensis, Brucella abortus of bovine and porcine origins, reported that Brucella melitensis is more like Brucella abortus isolated from swine. Such results seem rather inconsistent. Dr. Simpson mentioned the fact that he had found a positive blood serum on five women frequently aborting. I have examined tissues from about 50 cases of abortion and I have isolated Brucella abortus from one case. Kristensen has reported the recovery of Brucella abortus from a cystic ovary. It is interesting to note that the reports state that Brucella melitensis infection is more common in children than in adults. In this country Brucella abortus infection seems to be more prevalent in the adult.

Dr. D. Schuyler Pulford.—I would like to mention an experience I had with treatment of undulant fever. We have had seven cases of this disease in the Woodland Clinic. One had an associated periostitis to a quite marked degree. We had tried various treatments in other cases without avail so in this one we decided to give the patient a course of amidoxyl benzoate as used in the treatment of arthritis. With this treatment the periostitis disappeared. I think it worthy of consideration that you might cure this infection by the administration of a course of amidoxyl benzoate.

Dr. E. R. Mugrage.—There is just one question that came to my mind. We have noted the marked difference in every instance between the the papers given by Dr. Giordano and the discussion of Dr. Simpson and others. I wonder if it would be possible that the use of mechanical milkers on the one hand, and manual milkers on the other might explain the 90 per cent instance.

Dr. Walter M. Simpson.—"Contagious abortion of cattle" is a very bad term. In the first place, thousands of cattle that are not pregnant acquire Brucella abortus infection; in the second place, bulls have it; in the third place, many pregnant cows suffering from the infection do not abort. The question arises as to what constitutes an abortion-free herd. Merely to have no cattle in the herd that have a history of abortion is not enough. By use of the agglutination test one can determine which animals are reactors. But we know that there are instances in which the organism has been recovered from the milk and no antiabortus agglutinins were present in the serum. It is erroneous to say that the etiology of undulant fever is unknown. The etiology is most certainly known. The proof is conclusive that most of the cases of undulant fever in this country are the result of the ingestion of raw milk of unpasteurized dairy products. The important consideration is that a human being is sick as a result of an infection with some organism of the Brucella group acquired from raw milk. Just which one of the sub-groups is the actual invader is not so important from the clinical point of view. We must not miss the forest because one tree obstructs our view.

Dr. A. V. Hardy.—I am sure that you all understand that we did carefully consider the possibility of milk being the mode of spread. The packing houses in Iowa are however in cities of over thirty thousand and in these cities, particularly in Sioux City, practically all of the milk, 80 to 90 per cent, is pasteurized. We have found in our packing house workers that as a whole they are not heavy milk users. We have gone into that very carefully and the evidence seems almost conclusive, that milk played practically no part in the occurrence of these infections.

Dr. Charles M. Carpenter.—Most of the infection in milk rises with the cream. Cream and butter are infected more heavily than raw milk. Brucella abortus can live in butter for four months when stored at eight degrees C. We consider that raw milk is the chief source of infection but one may become infected through butter or cream.

MILK-BORNE RABIES*

By E. R. Mugrage, M.D., Denver, Colo.

RABIES is a disease which has been recognized for centuries, known under many names, and feared by all people. Its constant prevalence in these early days may be judged by the frequent mention made of it in the works of writers of those times. Since that time no period has been free of the disease, as mention can be found of its existence in each succeeding century. There have occurred ebbs and flows in its prevalence during these years. At times the disease has become epidemic, but usually localized in extent to one country, or section of a continent. Nothing is known of its primal origin, but it is spread world wide, except in portions kept free by quarantine regulations. The literature concerned with rabies is excelled by very few if any, of the other infectious diseases. But in spite of this, it is known to the vast majority of people as a vague entity of doubtful existence.

In the United States for the past five years, there has been an increasing amount of literature on rabies, in keeping with the growing prevalence in dogs and other animals. Current articles are appearing in public health bulletins, medical journals, and fortunately in a few of the popular magazines, which are more accessible to the general public. Increase in news items on rabies in the daily newspapers is also a very important way to bring the condition to the attention of the laity.

Due to the above propaganda, whether a man or animal is bitten, dog bites are apparently regarded of more consequence now, and the use of prophylactic treatment, both local and immunologic, is increasing.

No hesitancy is permitted in the use of rabies vaccine for prophylactic treatment of humans who have been bitten by known rabid animals, or who have come in contact with the saliva of such animals, while open wounds are present. Treatment in such cases is definitely advocated, and even insisted upon, by the great majority of medical men.

It is in the case of bites or the soiling of wounds by animals not known, or not traceable subsequently, that any question arises. Another angle to this problem is presented in the use of milk from cows which are rabid. Is rabies virus transmitted through the milk of these animals?

This question arises not infrequently, and a letter published in a recent medical journal[1] expressed questions of this kind. The answers given were not decisive, but the best possible with present knowledge.

Examples of a similar nature are (a) 21 people used milk from a rabid cow. All took rabies vaccine on medical advice. (b) Rabies developed in the herd of an institution, in which the milk was mixed. Treatment was

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From the Department of Clinical Pathology, University of Colorado School of Medicine, Denver.

not given. (c) A rabid bull in an institutional herd caused considerable controversy. Rabies, however, did not develop in the cows, but questions arose as to how the milk should be cared for, since it was used raw. (d) Four cows died of rabies in a herd, milk from which was mixed and sold to consumers.

Authorities are agreed that the virus is present in the saliva of the rabid animal, and that it may be present for several days before symptoms develop. Also that virus is present in the central nervous system, and nerve branches. This applies to both street and fixed virus. Virus in the saliva appears to be much more virulent[2] than the virus in brain tissue. A very small amount of the former is capable of producing infection, while it requires relatively larger amounts of brain tissue from the same animal to produce symptoms.

Opinion is not so unanimous for other body fluids and tissues. Blood,[3] it is stated, seldom if ever contains the virus. The flesh[4] of rabid animals, however, appears to be infectious. It is stated by Stimson,[5] and Mohler[6] who quote from several workers that the milk of rabid human beings and animals may contain the virus, however, very infrequently. Milk from a rabid bitch produced typical rabies when injected intramuscularly into rabbits, but the litter of pups removed from the mother escaped infection. A litter of pups under personal observation and under similar conditions has not developed symptoms in three months. Unfortunately the bitch was killed early, but the brain showed Negri bodies, and also produced experimental rabies in rabbits.

Limited search of the literature has not revealed any report of an authentic case of human rabies which has developed from the use of milk of a rabid cow, or other animal. A case of a human being cited[7] evidently shows the transmission of rabies to the child through breast milk. In another similar case[8] the child escaped. Examples of a like nature between cows and calves have apparently not been reported, although opportunities for such exposure must occur.

It appears that such positive evidence would prove that rabies virus can be transmitted through the milk. But the positive instances have occurred in animals in which the possibility for accidental contamination is marked. Also other sources for the infection must be considered. Man, dogs and cows during the course of the disease are apt to display a considerable degree of restlessness, irritability, parasthesias, and production of a large amount of viscid saliva. Observation of such a case shows how the entire body may possibly be contaminated with saliva. Some animals also are especially prone to lick themselves, and in that way a nursing animal may cover its breasts and nipples with living virus. In addition the mother kissing her babe, and the nursing animal licking the soft mucous membrane around the mouth of its young with its rough tongue, can easily account for minute abasions through which virus may enter. Milkers can very readily soil their hands with saliva on the animals, and in turn soil the teats and very probably contaminate the milk. Virus in dry saliva or dust may also gain entrance to the container, unless enclosed. Such factors as enumerated above, if true,

may lead to erroneous ideas of the source of the virus. A series of animal experiments were carried out with this in mind.

The rabbit seemed to be best suited for the work since rabies rarely ever develops except as the paralytic form. Also they seldom show drooling of saliva, nor do they lick themselves to the extent shown by other animals. The time of pregnancy is short, and inoculation of the animal can be closely controlled with the time of delivery. Also virus which will produce rabies in the rabbit should be virulent for other rabbits if present in the milk, while there might be a lessened virulence for other animals.

Three full-grown does were bred and four days before expected delivery, they were injected subdurally with brain emulsion from two rabid dogs. The brain substance from each dog had shown Negri bodies, and had also produced experimental rabies in rabbits. The animals were injected late to avoid possible placental transmission[9] which has been reported in animals. Also that the young might be almost weaned by the time symptoms set in.

The litters were allowed to suckle the doe almost to the time of her death. Restraint was required as a rule to permit the young to approach the breasts after symptoms ensued. With the onset of symptoms milk was also expressed from the several breasts daily, mixed and injected into the masseter muscle[10] of two half-grown rabbits. The above brain emulsion injected into the masseter muscle of control rabbits produced experimental rabies in twelve to sixteen days.

Neither rabbits of the litters nor those injected with milk from those that died, have shown symptoms of rabies. Nor has search for Negri bodies or subdural inoculation of brain tissue from these animals that died, been successful. Autopsy has with few exceptions shown a definite cause for death. Milk injections did not produce an abscess in any case. The remaining animals have grown, and are healthy after three months.

The results obtained in this small series of animals seem to exclude transmission of rabies virus through breast milk. This same view has been expressed by Heineman,[11] Keirle[12] and Nicolas.[13] But virus may be present from saliva. If present, in whatever form, it may potentially produce the disease. Gastric juice destroys the virus in approximately five hours, and it is highly improbable that intact mucosa of the gastrointestinal tract will permit it to pass into the tissues. Freezing is without effect, but heat will kill the virus. There is some disagreement on the thermal death point, Marx[14] reports forty minutes at 58° C., and van den Hoven van Genderen[15] shows that brain emulsions heated from 50° to 65° C. for fifteen minutes are still virulent.

All are agreed that boiling destroys the virus in a very few minutes. Drying is detrimental, but the time interval before virulence is lost is dependent on several factors; rapidity of desiccation, temperature, presence of oxygen and light; all influence the results. Oxygen seems especially detrimental, as virus in saliva drying in air and sunlight may be destroyed in fourteen hours,[16] while protected from oxygen, the virulence persists much longer. In milk, virus probably remains unchanged a long time, although no definite data has apparently been obtained. It is known that in the presence of moisture and

especially at ice box temperature virus keeps for weeks. Also virus is not destroyed early in putrefaction.[17]

The question has arisen of the efficacy of pasteurization of milk if rabies should be present. Regulations in most States require a temperature of at least 61° C. for thirty minutes. Experimental data is not certain that this is sufficient to destroy rabies virus.

Evidence is not clear that rabies can be transmitted through milk, except from extraneous contamination. Further evidence shows with rare exception that ingestion of known virus does not result in rabies in experimental animals, and the same results should hold for man. The question is whether rabies vaccine should be given in these cases.

There is the economic problem, for it would be a heavy expense to treat all individuals in an institution of any size, or even a small group of people who might partake of milk from a rabid cow. But treatment is indicated, if any risk is present. On this, authorities are inclined to be indefinite. Should some degree of risk be present, it is doubtful if it is as great as the development of posttreatment paralysis. This complication is not seen as often now, since the various modifications of the Pasteur treatment have apparently lowered the incidence. But it is still a problem in all parts of the world where any great number of the treatments are given, and the risk should be considered, as a long convalescence, or even death may ensue. Should a group of individuals be subjected to a definite risk, however small, to gain protection against an improbable infection?

SUMMARY

The general increase in rabid animals will bring up the possible transmission of rabies through milk more often in the future. Literature is conflicting on possibilities of such infection, and advice is indefinite on the necessity of treatment.

A small series of rabbits either injected with milk from rabid does or nursing these does did not develop rabies.

Data does not seem to justify antirabic treatment for individuals who have ingested milk from a rabid cow.

Pasteurization as ordinarily conducted may not destroy rabies virus present.

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MALIGNANT NEOPLASMS OF THE TESTIS*

By Osborne Allen Brines, M.D., Detroit, Michigan

MALIGNANT neoplasms of the testis, while not rare, are not common. About 700 cases have been recorded in literature. Several times that number have probably been actually observed. On two fairly large surgical services I have found that about three cases a year is their average. The present status of our knowledge on the subject is more or less chaotic, and yet, it is rapidly improving and is considerably more satisfactory than only a few years ago when nearly all of the surgeons as well as some pathologists considered nearly all of these tumors as being sarcomas.

The present opinions on the subject are well known and can readily be divided into two groups: (1) the following, headed by Ewing[1] who believes that all malignant tumors of the testis are teratomas, and (2), the group composed principally of Chevassu,[2] Schultz and Eisendrath,[3] Southam and Linell,[4] Tanner,[5] Bell[6] and others who take the position that, while teratomas constitute a large group of these tumors, there is an equally large and important group of tumors composed of homologous epithelial cells and presenting no evidence of teratomatous elements. These pure epithelial tumors are composed of cells which are usually described as being large spherical cells with clear cytoplasm and large vesicular nuclei. Because of their resemblance to the epithelial cells lining the seminiferous tubules, those concerned with spermatogenesis, these neoplasms have been called seminomes or spermatocytomas.

Ewing's opposition to the existence of such a growth has been that they were only apparently homologous, an instance either of failure to examine the tumor with sufficient care or of one type of cell proliferating to the nearly or entirely complete obliteration of other cells originally present.

Not long ago such a view was considered by many absurd, requiring a

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From the Departments of Pathology, Receiving Hospital and Detroit College of Medicine and Surgery.

play of imagination and to say the least, extreme open-mindedness. Today one can appreciate the wisdom of Ewing's position and the fearlessness necessary to be the first to express such a viewpoint because the theory is necessarily somewhat vague and the proof not easily demonstrated.

The basis of this paper is a review of 32 cases, collected during the past ten years. First, merely the existing slides were reviewed. Later, from six to fifteen more blocks were taken from each specimen. Then in each case the second diagnosis was compared with the one originally made. The original diagnoses were interesting and amusing, ranging from spermatocytoma to teratoma and including lymphosarcoma, round cell sarcoma, various types of carcinoma, and malignant adenomas.

It was interesting to find that on reviewing the original slides on the basis of an accepted and apparently reasonable classification (the most rea-

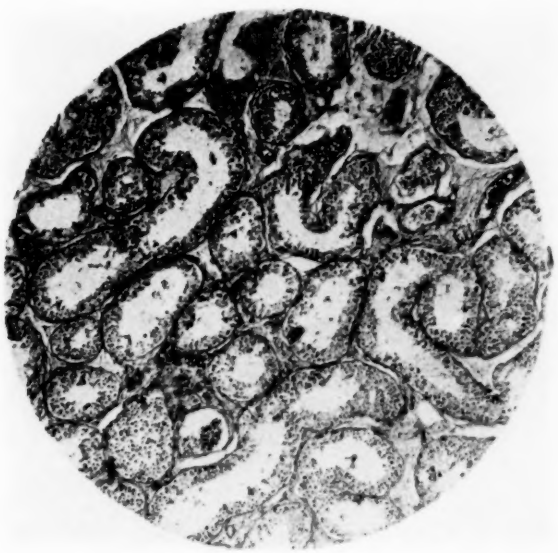

Fig. 1.—Essentially normal testis.

sonable I thought at the time), there were exactly sixteen teratomas and sixteen spermatocytomas or pure carcinomas (homologous epithelial tumors). But when the slides were examined from the six to fifteen additional blocks from each specimen, it was necessary to remove six cases from the spermatocytoma group into the teratoma group, leaving twenty-two teratomas and ten tumors which revealed no heterologous elements. This proved the correctness of Ewing's contention. Sufficiently careful examination had detected heterologous elements in all but ten cases where probably the number of sections was still insufficient or where there was an extensive overgrowth of one type of cell. The important point is that by the examination of a large number of slides it was possible to make a diagnosis of teratoma where a small number of sections from each specimen furnished insufficient evidence to make such a diagnosis.

Teratomas are not any too thoroughly understood and the teratomas of

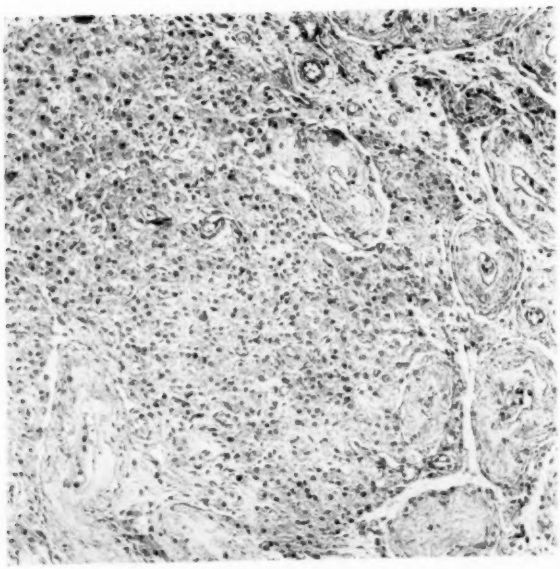

Fig. 2.—A small malignant area in undescended testicle.

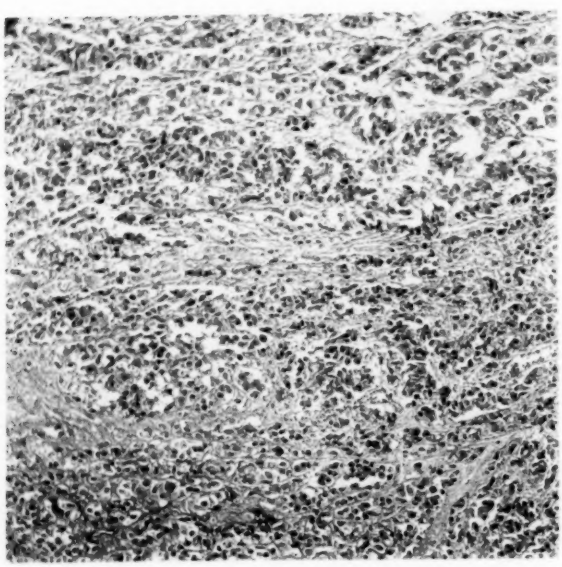

Fig. 3.—Typical newgrowth area composed of germinal cells.

the testis are a special problem. At a very early stage in the formation of the mesonephros, a narrow strip of mesothelium extending along the medial surface becomes thicker and the cells become arranged in several layers. This is the germinal epithelium which is composed of two kinds of cells: (a) small cuboidal cells with rather intensely staining cytoplasm which become interstitial cells; and (b) the large spherical cells with clear cytoplasm and larger vesicular nuclei referred to above and which are the sex cells, destined, in the male, to give rise to spermatozoa.

The germinal cells are important in the histogenesis of malignant tumors of the testis. Their appearance is characteristic, they are easily recognizable and they were present in every case in this series. They are epithelial cells derived from the mesoderm. It is easy to see how the term "embryonal carci-

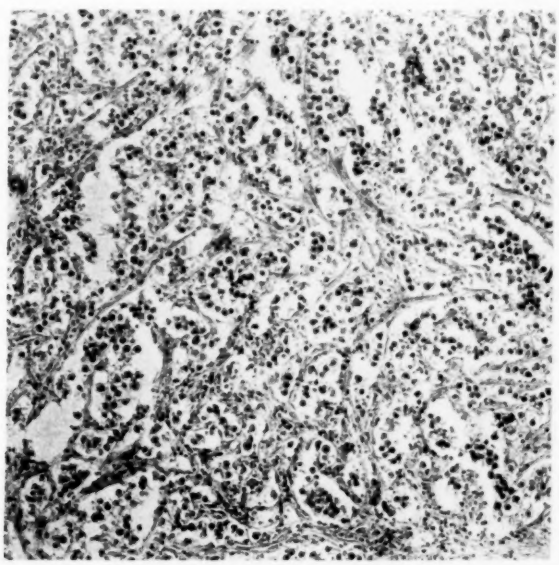

Fig. 4.—Newgrowth area with pattern suggestive of normal testis.

noma" came into usage, because in spite of the bidermal or tridermal nature of the tumor, these characteristic epithelial cells are always or usually present. The presence of cartilage in newgrowths of the ovary or testis is usually interpreted as evidence of a mixed tumor and yet cartilage with these typical germinal cells would, strictly speaking, be only a monodermal tumor, all cells being derived from the mesoderm; however, I have never found cartilage in these tumors in the absence of both ectodermal and endodermal derivatives.

These typical cells which have served to confuse and complicate the interpretation of these teratomas have quite a wide range of variation of morphology. Schultz and Eisendrath[3] believe they can recognize the different stages of spermatogenesis which these cells represent, but that is doubtful. Variation in the morphology of cells composing tumors of any organ is common. Appreciation on the part of the microscopist of the variation in cell arrangement is most important when attempting to make a diagnosis of a

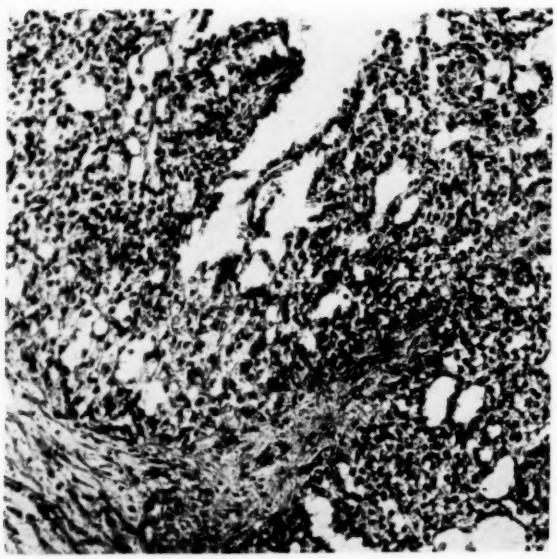

Fig. 5.—Germinal type cells with papillary arrangement.

Fig. 6.—Teratoma of testis with definite papillary arrangement of epithelial cells.

neoplasm of the testis. The cells frequently grow in a solid encephaloid formation with a marked tendency to necrosis (Figs. 2 and 3). Again they assume an alveolar arrangement which suggests a definite relationship to the tubular epithelium, just as malignant cells elsewhere bear a resemblance to the normal cells to which they are related (Fig. 4). But unfortunately they also assume a bizarre alveolar arrangement, in no way suggestive of normal testicle, sometimes with a marked papillomatous arrangement (Figs. 5 and 6). Most confusing of all is when these cells become so atypical in morphology and arrangement that they cannot be distinguished from highly malignant cells of endodermal derivation (Fig. 7). I believe it is often impossible to say definitely when examining these tumors microscopically whether certain cells are germinal or endodermal in derivation.

Fig. 7.—Newgrowth cells of doubtful derivation.

The malignancy of these tumors is either wholly or in part due to the germinal type cells and metastases usually consist of these cells. However, the cells of endodermal derivation can and do become highly malignant but the malignancy of tumors of the testis is invariably due to epithelial cells.

The prevalence of germinal epithelial cells in teratomas of the testis has been explained by Ewing as the result of a one-sided development. That hardly accounts for the uniformly consistent presence of these cells. Why should differentiation proceed so unvaryingly in this one direction?

Other authors[11] have suggested another explanation which, on the surface at least, sounds quite reasonable. The sex cells at an early stage are totipotent, i.e., capable of producing any order of cell in the body. Therefore, a mixed tumor might arise from misplaced germ cells forming rests which proliferated later with one type of cell possibly becoming the most aggressive, resulting in the formation of an apparently homologous tumor.

It should be remembered that small cysts, frequently in the capsule must

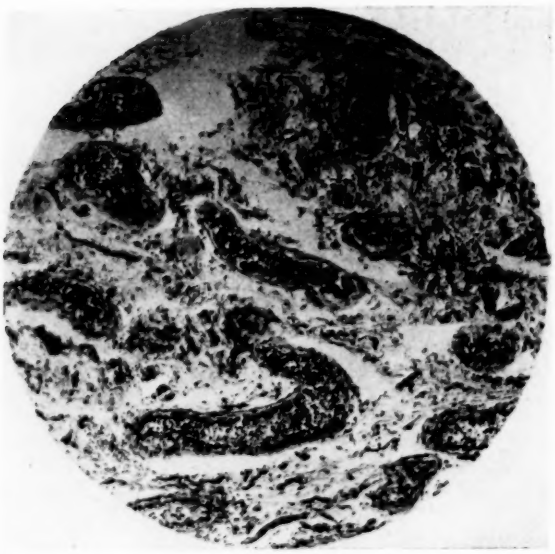

Fig. 8.—Newgrowth cells extending through adjacent seminiferous tubules.

Fig. 9.—Typical metastatic area in lung.

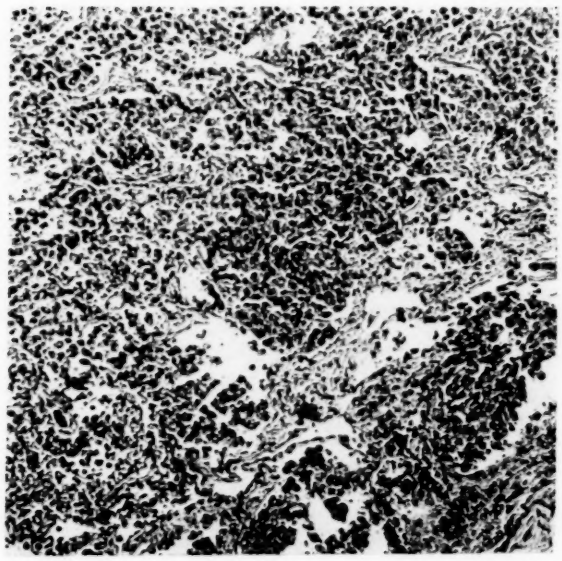

Fig. 10.—Newgrowth cells resembling interstitial epithelial cells of testis.

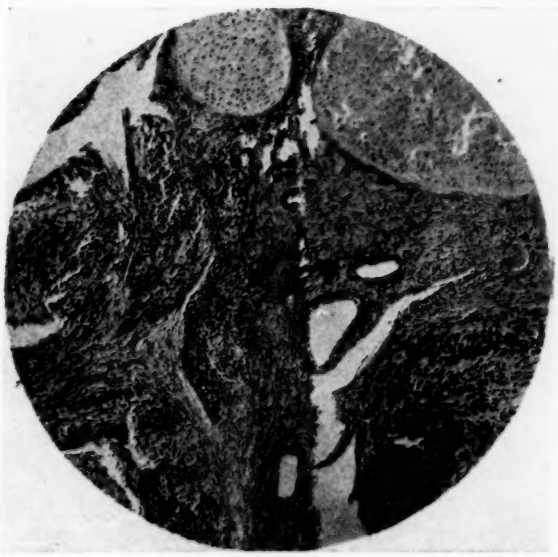

Fig. 11.—Different types of epithelium associated with cartilage in a teratoma of the testis.

not be confused with endodermal derivatives but are probably müllerian duct derivatives.

From a careful study of the slides from these cases it seems apparent that the newgrowth cells extend to adjacent testicular tissue by way of the seminiferous tubules. It is not difficult to find areas in which the newgrowth cells fill the tubules for a full low power field or more beyond the growth in the intertubular stroma. (Fig. 8.)

The material studied furnished evidence that not only the tubular epithelium but also the interstitial epithelium were closely related to the newgrowth. This is not surprising when the embryology of the testis is considered. In one case there were extensive metastatic areas in the liver, lungs, and retroperitoneal lymph nodes (Fig. 9), but numerous sections of the testis showed only one suspicious area consisting of hyperplastic cells resembling interstitial epithelium and histologically not malignant.

In another case the metastatic areas in the liver consisted largely of cells which closely resembled the interstitial epithelial cells of the testis (Fig. 10). A paper is now being prepared to present the theory that a constant transition takes place between the intertubular cells and the interstitial epithelium in the same way that this interrelationship has been claimed to exist between acinar and islet cells of the pancreas by Otani,[7] between the parenchymal and reticular cells of the thymus by Gottesman and Jaffe,[8] between the liver rod cells and bile duct epithelium by MacCallum[9] and between the acinar and interstitial cells of the thyroid by Hertzler.[10] According to some embryologists the interstitial epithelial cells are derived from sex cells and according to others they come from the interstitial connective tissue.

SUMMARY AND CONCLUSIONS

1. The histopathology of tumors of the testis is often confusing and frequently misleading so that an accurate classification is difficult to arrive at.

2. The fact that, by the examination of additional sections in each of the thirty-two tumors in this series it was possible to transpose six cases from the homologous tumor group to the mixed tumor group, is valuable proof of the correctness of Ewing's position.

3. However, Ewing's explanation that the almost pure epithelial nature of these tumors is due to a one-sided development of a teratoma does not appear as reasonable as to say that these tumors arise from very young sex cells which are still totipotent and are therefore capable of giving rise to heterologous elements. Additional proof for such a view lies in the established fact that mixed tumors of the testis are more malignant than the so-called spermatocytomas which could be construed to mean that mixed tumors are derived from very young germ cells, capable of producing ectodermal and endodermal tissue, whereas the homologous nature of some of these tumors may be due to the misplaced germ cells taking on newgrowth characteristics after their totipotent limitation had been reached.

4. While I think that the proper conception of these tumors is that they are all mixed tumors, I am not sure teratoma is a good term. They do not possess many of the characteristics of teratomas. It is difficult to think of

them in a parasitic sense and besides they are so predominatingly epithelial in composition. Possibly embryonal carcinoma is preferable.

5. Definite proof cannot be offered to bring about the sudden acceptance of the view that malignant tumors of the testis are universally mixed tumors, yet pathologists, who in their daily routine work considering the subject carefully, will doubtless be slowly but surely converted to this view.

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2201 Jefferson Avenue E.

RETICULOCYTES: THEIR IDENTIFICATION AND SIGNIFICANCE*

By Carl L. Spohr, M.D., Columbus, Ohio

THE red corpuscles of the blood have been extensively investigated. They are free in the blood stream and are easily available for study. They are unique in that they normally function after the loss of the nucleus. Key[1] says mature erythrocytes have lost their cytoplasm and are not living cells. Harrop[2] observes that the normal mature erythrocytes have no measurable oxygen consumption. The nature of the internal structure has received considerable attention. The erythrocyte may be considered as a balloon containing a stroma and a functioning substance as a relatively fluid colloid. The hemoglobin part may be in the state of a hydrophilic gel. The covering consists of a lipoid and protein stroma. Ultramicroscopic examination of the erythrocyte fails to reveal anything as to its structure. The number of erythrocytes shows considerable variation as does the hemoglobin. The controversy as to the single or multiple origin of blood cells need not concern us, except that Ehrlich[3] advanced the polyphylectic theory while the monophyletic theory is advocated by Pappenheim,[4] Maximow,[5] Danschakoff.[6] Sabin quoted by Woollard,[7] more recently, has given a detailed account of the morphologic changes that occur in the various cellular forms that precede the formation of the red corpuscle in the chick. From the common mesenchyme cell is derived the angioblast. These basophilic cells form syncytial masses, sprout to form plexuses, and form primitive blood vessels and primitive blood cells. Red blood cells actually arise from the endothelium and the primitive angioblast. We have no knowledge of the factors which induce the primitive mesenchyme cells to form the endothelium of the first capillaries.

The youngest representative of the red cell, according to Woollard,[7] is a fairly large round cell with only a faint trace of hemoglobin in the deeply basophilic cytoplasm, with a large vesicular nucleus. They show large rods (mitochondria?) by super vital staining with Janus green B. This cell and its immediate offspring may be considered as megaloblasts. They are an indication of active erythropoiesis and are seldom seen in normal bone marrow. They are followed by the erythroblast, also showing rods, more hemoglobin, and a denser nucleus. The nucleus becomes more pyknotic and is finally extruded. The cytoplasm becomes apparently homogeneous and the granules and rods disappear entirely. We now have the adult (senile) erythrocyte ready to begin its life's work. How long this cell continues to function is not definitely known. It has been discussed by Rous.[8] It is estimated that one-fifteenth of the erythrocytes of the body are destroyed daily. The average life expectancy, therefore, is about fifteen days. Quincke estimates it at thirty days. Ashby[9] says, "Estimates vary from two to four weeks." The useless cells are quickly removed from the circulation, but the fate of the erythrocytes is, by no means, a settled question (Rous[8]).

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Vital staining consists in mixing a drop of freshly drawn blood with a bit of stain and observing the blood before it is dried. Ehrlich,[3] using methylene blue in this way, found in the cytoplasm of some of the cells a bluish network. Pappenheim,[10] using neutral red, observed granules in anemias. Ceasris-Demel, using cresyl violet, observed granules, filaments, and networks. In Europe these cells were called "granulo-reticulo-filamentous cells." In America they have been called "reticulated cells" and Krumbhaar[11] suggested the word "reticulocyte" and regarded an increase in the number of these cells as a "reticulosis." Adult human blood contains from 0.1 per cent to 1 per cent, while in infants, during the first week of life, the reticulocytes show a much higher percentage. At the end of the first week, the blood begins to resemble the adult picture. Physiologically, all are agreed that the reticulocytes are young, growing red cells.

Key[1] has studied reticulosis in considerable detail, and his results are briefly given: When a drop of blood is mixed with brilliant cresyl blue, the reticulum is quickly stained a purple. A moss-like wreath is the usual form; but all gradations, ranging from cells containing a few basophilic granules or fragments, through rather coarse networks and loosely constructed wreaths, can be seen in the same blood. In addition to these cells, one often sees cells which stain diffusely. In nucleated red cells of embryonic blood, the reticular network surrounds the nucleus. An occasional nucleated red blood cell is found in which no reticular substance can be seen. Azur II stains the reticulum blue; thionin stains the reticulum blue; methyl green does not stain the reticulum. It can also be stained with many other basic dyes. Janus green B, used extensively as a specific (?) supravital stain for mitochondria, will stain certain of the erythrocytes a faint diffuse green color. A variable number of small green granules and rods appear, connected by delicate green strands so that a definite reticulum is formed. This green color gradually fades leaving the reticulum as a refractile network. The percentage of erythrocytes containing reticulum in a preparation stained with Janus green B corresponds closely with the percentage of reticulated cells in a preparation of the same blood stained with brilliant cresyl blue. Cowdry[12] was able to stain mitochondria in lymphocytes with a solution of 1:500,000 Janus green B. In order to stain the reticulum in the erythrocytes, much stronger solutions are necessary. A concentration of 1:6,000 was found satisfactory.

Air dried smears of anemic blood, stained with any of the stains which stain reticulum supravitally, fail to stain the reticular substance in wreath-like form, but as basophilic fragments and granules; and there is no diffuse staining.

In true polychromatophilia the staining is diffuse and no granules or fragments are seen. In Wright's method after alcohol fixation and staining the picture is one of polychromatophilia, but if the Wright stain be first allowed to dry on the slide, and the staining is done by the supravital method, a definite basophilic reticulum appears.

It has long been known that a basic staining substance is present in certain erythrocytes and that it can be demonstrated in the form of polychromatophilia, punctate basophilia, or reticulum by appropriate staining

methods. It is quite generally believed that these basophilic forms are young erythrocytes. In the circulating blood of normal adult human beings no polychromatophilia or punctate basophilia and not over one per cent of reticulated red cells are found. Basophilic erythrocytes are commonly found in adult bone marrow. In anemic adults the percentage of basophilic erythrocytes is roughly proportional to the activity of the bone marrow.

Hawes[13] concludes that polychromatophilia, stippling, and reticulation are all different manifestations of the same process.

The addition of oxalate to the blood does not seem to affect the reticulum of the erythrocytes; and the reticulum can still be stained.

Schilling-Torgau[14] states that the reticulated erythrocytes, treated with very dilute alkalies, and then stained supravitally, give pictures resembling punctate basophilia, and he considers this as a transition stage between reticulation and polychromatophilia.

If we consider erythrocytes which contain basophilic substance as young erythrocytes, these cells are slightly larger than mature cells (Hawes[13]) and apparently contain a lower percentage of hemoglobin. Reticulated cells exhibit a tendency to agglutinate. The specific gravity of reticulated cells is slightly lower than that of normal erythrocytes. The reticular substance is not soluble in water or in sodium chloride solution, and in blood which is laked in distilled water, the refractile granules are not dissolved and can be seen stained in the usual manner. (Key.[1]) The reticular substance is not soluble in ethyl or methyl alcohol, and upon the addition of 5 per cent acetic acid, reticular substance can still be seen in a fragmented form. The reticular substance appears as refractive granules in solutions of various acids, and is insoluble.

That the presence of basophilic substance in erythrocytes is evidence of youth is indicated by the fact that basophilia and reticulation in erythrocytes is increased in states in which blood formation is stimulated, and decreased in states in which it is inhibited; not only in clinical states, but also in experimental conditions.

Basophilic cells are present in increased numbers in embryonic blood, and their percentage progressively decreases as the embryo approaches term. They are also present in large numbers in bone marrow and in embryonic livers where blood formation is in progress, and the basophilic substance is demonstrable in nucleated red cells, and can be seen to remain in the cell as the nucleus is being extruded.

According to Key,[1] the basophilic substance, which he considers a better term for reticulum, does not appear in any other cells of the body. In no other cell is the protoplasm replaced by hemoglobin, and in no other cell is the nucleus lost.

The state in which this basophilic substance exists in the unaltered erythrocyte is unknown. Apparently either the diffuse polychromatophilia or the reticular net is due to the technic used.

The reticular substance may be distinguished from the nucleus by differential staining, and it is believed that the reticular substance is of cytoplasmic origin. It may be a remnant of the protoplasm which is ultimately replaced by the hemoglobin.

METHODS OF STAINING

There are a number of methods in use for demonstrating the reticular substance of erythrocytes. Brilliant cresyl blue has been extensively employed. The blood can be added to a solution containing about 0.2 per cent of brilliant cresyl blue and 1 per cent neutral potassium oxalate in 0.85 per cent sodium chloride solution; allow it to stand from ten to twenty minutes; centrifuge to precipitate the corpuscles; transfer the sediment to a clean slide; air dry; and examine with the immersion lens. The percentage of reticulocytes can be calculated after examining at least three thousand cells for reticulation. An ocular micrometer disc, suitably ruled, greatly facilitates counting.

A method which is more frequently employed and which in our hands has given excellent results is one in which scrupulously clean covers or slides are used, the surface of which is covered with a thin layer of the dye. These are prepared by placing on the slide a drop of 0.5 per cent alcoholic solution of brilliant cresyl blue, spreading it and allowing it to dry in the air; or drying can be hastened by passing the slide through a flame. It is essential to avoid the collection of dust on the surface of such smears. A drop of blood is placed on the slide which is covered with the thin film of dye, and a clean cover glass is dropped on. The preparation is ringed with vaseline. After allowing one minute for staining, the counting can proceed.

A modification of this method is to omit the ring of vaseline; and, after the lapse of one minute, raise the cover glass gently, spread the drop of blood with the cover, air dry, counterstain with Wright's method, and enumerate as before mentioned. Or a cover glass filmed with the dye can be used in a similar manner. In the wet preparations ringed with vaseline, it is possible to enumerate blood platelets at the same time, as they are tinged a faint lavender color.

The number of reticulocytes is usually reported in percentage, or the total number per cubic millimeter can be reported. By so reporting we can quickly note the total number of reticulocytes as compared with the average number found in normal blood. Furthermore, valuable information is obtained by comparing the total number of reticulocytes present in the blood at a given time with the total number of cells present in the blood of the patient. It is also of value to compare the total number of reticulocytes per cubic millimeter in successive counts.

CLINICAL SIGNIFICANCE OF RETICULATED CELLS

The reticulocyte is universally considered to be an immature red blood cell, but the nature and meaning of the reticular substance, the relation which it bears to the nucleus, and the mode by which the nucleus is lost to the cell are still disputed. The presence of normoblasts in the normal red marrow and the occurrence of reticulocytes in small numbers suggests that in all probability they are the normal stages in the evolution of the anuclear cell. The characteristics of the nuclear material and the characteristics of the reticular or basophilic substance indicate that the reticular substance is probably of

cytoplasmic origin and a remnant of the protoplasm remaining in the cell when replaced by the hemoglobin.

The reticulocyte is considered a fair index of the activity of the blood-forming organs, especially in the anemias which respond to liver therapy.

Murphy, Minot, and Stetson[15] in a report on the response of the reticulocytes to liver therapy find a high percentage in pernicious anemia. In pernicious anemia the anemia is primarily dependent on the failure of the primitive cells in the bone marrow to differentiate toward mature cells. In pernicious anemia they found regularly a prompt, temporarily pronounced increase in the number of reticulocytes in the peripheral blood following the administration of large amounts of liver, kidney, or potent fractions of liver, the number remaining elevated for about nine days. The height to which the percentage of reticulocytes rises is in inverse relation to the number of red cells; cases with more than three million never exhibit more than a slight increase in reticulocytes. Accompanying the reticulocyte response other immature forms of bone marrow elements may appear in the blood; also an increase in white cells and platelets. They also found that feeding large amounts of liver produces no response of the reticulocytes in normal persons. A similar response does not hold for secondary anemias; it is absent or very slight in these cases. Some unusual cases and some cases of anemia associated with pregnancy have shown a marked response of reticulocytes similar to that found in pernicious anemia. This has also been observed in sprue and in anemia due to the fish tapeworm.

With dietetic treatment an improvement was noted in most of the cases in a short time; when the erythrocyte count reached a certain level, the increased number of reticulocytes diminished. Huth and Kessler[16] report an advantage in the use of liver extract in that it is possible to increase the dose without objection on the part of the patient; when whole liver was given they found that they obtained more satisfactory results by varying the form and the method of serving. Their first indication of a favorable effect of the liver diet was the rosy appearance of the skin which Pal attributes to the glow of the blood under the tissues still transparent as a result of the anemic condition. They also noted that there is a failure of corpuscular formation to parallel the formation of blood pigments; pernicious anemia is a hyperchromatic anemia; with improvement there is an increase in red cells and a gradual fall in the color index, indicating that the number of red cells and the hemoglobin do not increase at the same rate. The pathologic aspect of the blood picture as far as the form of the red cell is concerned disappears with successful treatment.

William Dameshek,[17] in his report of 50 cases of pernicious anemia, finds it an ideal disease in which to study blood changes with corresponding variations in the reticulocyte count, over long periods of time. He observed an increase in the reticulocyte count in 80 per cent of the cases. The increase was at least 5 per cent and occurred before a remission. In 96 per cent of the cases the reticulocyte count fell, or remained low during a relapse. Increases of 6 per cent or more may with some degree of certainty be considered as presaging a remission. In nearly all cases there was some rise, however slight,

following a transfusion. Not uncommonly he found that when a remission was unlikely, because of low reticulocyte count, transfusion caused apparently the necessary stimulating effect and there followed a marked increase in the reticulocyte count and a remission occurred.

He also noted that polychromatophilia, an accepted evidence of regeneration in the fixed preparation, runs parallel with the reticulation of the unfixed smear, at times.

In a few cases of aplastic anemia observed, a condition representing a rapid failure in bone marrow growth, the reticulocyte count is found to be almost nil and remained so until death.

In purpura hemorrhagica, in which there is a failure in the platelet growth resulting in hemorrhage and anemia, it was observed that when the reticulocyte count was low and remained so, the patients continued to bleed and finally died. When the reticulocytes increased, the platelets increased and the patient recovered.

He also found that in congenital hemolytic anemia, marked reticulosis is pathognomonic, differentiating that disease from other anemias associated with large spleens.

In a report by W. W. Duke,[18] aplastic anemia, originally described by Ehrlich, is caused primarily by aplasia of the bone marrow. As a sequence the rate of blood regeneration is reduced and anemia inevitably follows. The symptoms displayed by a patient with aplastic anemia depend on which of the formed elements of the blood are most markedly reduced. If the red cell reduction dominates the situation, anemia is the prominent symptom. If the thrombocytic element is the most markedly involved, the prominent symptoms are hemorrhage or purpura hemorrhagica. It is generally accepted that the agent that injures the marrow also tends to destroy the formed elements in the blood itself. This may give a blood picture similar to that of pernicious anemia.

The type of severe anemia generally called "aplastic" anemia because the bone marrow in such a case makes little or no effort to regenerate the blood cells is at times caused by known agents, and is then known as "secondary aplastic" anemia; when the cause is unknown the term "idiopathic aplastic" anemia is applied.

The blood picture reflects the status of the bone marrow as seen at autopsy, and Rennie believes it due to a congenitally weak bone marrow, which at an early age becomes unable to carry on the function of blood formation.

Murphy and MacEachern[19] discussed the differentiation between aplastic and pernicious anemia. Ordinarily the diseases that are confused with aplastic anemia are pernicious anemia, purpura hemorrhagica, and myelophthisic anemia. Aplastic anemia with secondary purpura may be difficult to differentiate from idiopathic purpura hemorrhagica. Minot[20] believes that there may be a relation between the two conditions and describes what he calls intermediate cases. In some cases (purpura hemorrhagica) the bone marrow cannot respond to the stimulus received by hemorrhage as it should, and the white and red elements of the bone marrow may have been depressed.

Minot also claims that the aplasia may involve the platelets markedly, white cell elements slightly, the red cell not at all, and regenerative forms may be numerous.

In idiopathic purpura hemorrhagica the white cell count is usually increased and the red cell count indicates the normal response on the part of the red cell centers to furnish erythrocytes to the blood stream which has lost many cells through hemorrhage resulting from a thrombopenia. A decreased platelet count is first noted and a decrease in the red blood corpuscles and white blood corpuscles follows. In true idiopathic aplastic anemia red blood corpuscles and white blood corpuscles decrease first and a platelet decrease follows, anemia before hemorrhage.

According to Dyke,[21] the reticulocyte count is a very important means of observing whether or not a patient with pernicious anemia will respond to a liver diet. Counts should be made daily in order to avoid missing the crisis, which should reach its maximum in about a week and may be as high as 30 per cent reticulocytes. Reticulocyte crisis is apparently explained as suggested by Peabody, who pointed out that in the relapsing stage of pernicious anemia the bone marrow is packed with red cells of immature megaloblastic type which appear incapable of completing their development. If thrown into the circulation in this state, they are rapidly destroyed. He suggests that the action of the liver is to supply a stimulus enabling these cells to reach maturity. The reticulocyte crisis may be looked upon as representing an act of catharsis on the part of the bone marrow, after which cell production proceeds in a normal manner.

In aplastic anemia, if the platelets fall below 20,000 to 60,000, purpura hemorrhagica appears and may give rise to a profuse hemorrhage from the nose, gums, gastrointestinal tract, bruises, etc. Petechiae follow slight injuries. With this symptom complex goes the finding of normal coagulation, prolongation of the bleeding time, firm, but nonretractile clot, and petechiae. Duke finds that transfusions are useful in restoring the red cells and platelets which may be of several days' duration, and also finds that diet is of less value than in pernicious anemia.

Doan[22] suggests that the suppression of red blood cells and hemoglobin formation are due, not to lack of substances in the liver, but to an excess of some inhibiting substance. It is suggested that the factor of inhibition or suppression of blood formation in the megaloblastic stage may be a beneficial factor in liver diet treatment of pernicious anemia.

Ashford[23] says that the anemia of sprue yields a shower of reticulocytes when liver extract is administered, unless the bone marrow is aplastic. Isaacs, Sturgis, and Smith[24] state that the type of young or new red blood cell delivered into the peripheral circulation depends on the stage to which the erythroblastic tissue in the bone marrow has developed. When the bulk of the cells are in the nucleated (megaloblastic) stage, the first evidence of stimulation by liver therapy is increased development to the next stage (reticulocyte). When the bulk of the cells in the bone marrow are in the latter stage, stimulation is followed by the increased production of mature cells. Many other writers, Middleton, W. S.,[25] Whipple, G. H.,[26] West, R., and Nichols, E. G.,[27]

and Means and Richardson[28] consider a reticular response as evidence of the value of liver therapy. Light is thrown on the relative age of a given erythrocyte by the appearance of its reticulated substance. (Ceasris-Demel.) While the percentage of reticulocytes in the circulation is raised whenever the new blood cells are being formed in increased numbers, it by no means follows that the percentage of reticulocytes is commensurate with the degree of anemia. The response depends on the condition of the bone marrow, whether it is healthy or "damaged." It is much more marked in hemolytic jaundice than in pernicious anemia.

Berglund, at a meeting of the American Association of Physicians held at Washington, D. C., in May, 1928, read a paper on the use of fetal liver in anemias. At the Ohio State University Hospital there is a patient who was admitted in April, 1927, suffering from idiopathic aplastic anemia. Until August, 1928, this patient was intensively treated with liver and liver extract. During this period he had frequent hemorrhages and, in spite of numerous blood transfusions, showed a persistently low blood count. In August, 1928, fetal liver, given raw, and in quantities varying from 600 to 800 grams daily, was employed with rather striking results. The reticulocytes, previously absent, appeared in the blood stream during September, and by December, 1928, his blood showed 10 per cent reticulocytes. Since that time there has been a gradual decline in the percentage of reticulocytes. During April, 1929, the red cells reached a total of 3,300,000 while at the beginning of the fetal liver therapy the total number of red cells was only 1,300,000. The hemorrhages are much less frequent and only occasional blood transfusions have been indicated. This case was reported by Upham and Nelson, *Fetal Liver Feedings in Aplastic Anemia*, at a meeting of the Missouri State Medical Association in May, 1929, and shortly will be published.

SUMMARY

Reticulocytes are immature erythrocytes containing, within the cytoplasm, a basophilic substance, which, by means of vital staining, can be demonstrated in the form of a reticulum.

Reticular substance is not present in the adult erythrocytes.

The addition of oxalate to the blood does not interfere with the action of dyes which stain the reticulum.

The most suitable vital stains for reticulum are brilliant cresyl blue, or a suitable methylene blue.

With the introduction of liver therapy in pernicious anemias, the enumeration of reticulocytes is a valuable procedure. Inasmuch as it is a satisfactory method of noting the results of treatment, reticulocyte counts should be made daily to determine whether a given case will respond to liver therapy, and in order to avoid missing the reticulocyte crisis.

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THE DETERMINATION OF HEMOGLOBIN WITH THE PHOTOELECTROMETER*†

By Arthur H. Sanford, M.D.,‡ and Charles Sheard, Ph.D.,§
Rochester, Minn.

A YEAR ago we reported a new method for the estimation of hemoglobin.[2] The apparatus as described has been modified and simplified during the last year. A detailed description of these changes will be presented in another paper.[3] We also propose the name photoelectrometer for the apparatus. Briefly, we may state that the chief changes consist in the substitution of a micro-ammeter in place of the galvanometer as the measuring device, and the introduction of one stage of amplification using an ordinary vacuum tube. The housing has also been improved, making the instrument more portable and less liable to outside disturbances. Many of the mechanical improvements have been made by Mr. Dana Rogers, of the Section on Biophysics.

With the changes indicated we have spent many weeks in calibrating the apparatus for oxyhemoglobin and in making new curves and tables. An attempt has been made to standardize all procedures so that the instrument may be easily used for routine work. We shall not present protocols of our various series of experiments, but rather present briefly those steps that we now deem necessary for the proper use of this method. Besides the assembling of parts in the metal housing and the micro-ammeter, it is necessary to use batteries as follows: one 6 volt "A" battery, three blocks of 22.5 volt "B" battery, one 4.5 volt "C" battery, and one 1.5 volt "dry cell."

Some of the connections to these batteries are made permanently, and others must be opened whenever the apparatus is left at rest for any length of time.

PERMANENT CONNECTIONS

1. Three 22.5 volt "B" batteries are connected in series, thus making a 67.5 volt "B" battery.

2. The negative (–) terminal at the "B" battery is connected to the negative (–) terminal of the micro-ammeter (Fig. 1).

*Read before the Eighth Annual Convention of the American Society of Clinical Pathologists, Portland, Oregon, July 5, 6 and 8, 1929.

†Since the preparation of this paper we have found that Stanley P. Reimann of the Research Institute of the Lankenau Hospital, Philadelphia, has published a preliminary report on the photoelectric cell as a colorimeter. This preliminary report was made at the New York meeting of the Society for Experimental Biology and Medicine, April 21, 1926.

Patent applications are pending which cover the use of photoelectric cells in the measurement of hemoglobin and the general principle of the applications of such methods with selective spectral filters to the determination of the amounts of unknown substances in solution, both with and without various accessories, such as one or more stages of amplification and various detecting devices. These applications for patent rights have been made in order to control the development, accuracy and general service-ability of such devices, so that those who acquire apparatus involving the principles disclosed may secure satisfactory equipment. Assignment of any rights granted will be made to the American Society of Clinical Pathologists.

‡Section on Clinical Laboratories, The Mayo Clinic.

§Division of Physics and Biophysical Research, The Mayo Foundation.

3. Another lead from the negative (−) terminal of the "B" battery is connected to the positive (+) terminal of the 4.5 volt "C" battery through a resistance of approximately 3000 ohms.

4. The positive (+) terminal of the 1.5 volt "dry cell" is connected to the binding post on the instrument marked "1.5 +" (Fig. 2).

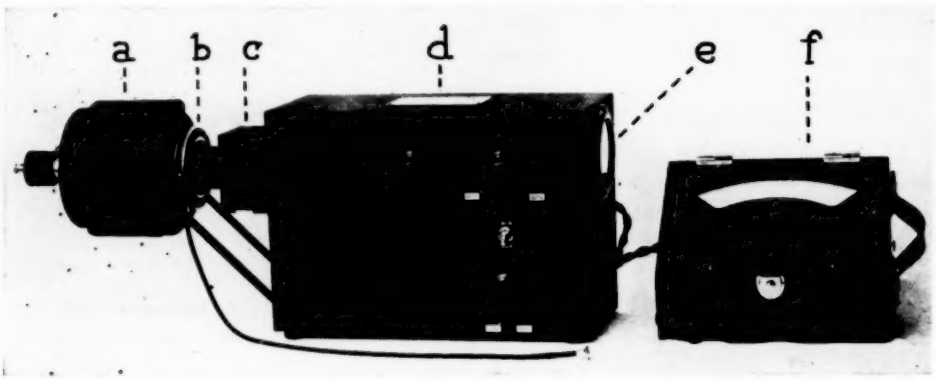

Fig. 1.—The photoelectrometer. *a*, Lamphouse and sources of illumination; *b*, adjustable iris diaphragm; *c*, receptacle for holding diluted sample of blood and selective spectral filter; *d*, container for photoelectric cell and one stage of amplification; *e*, meter for reading current in illuminating circuit; *f* micro-ammeter for reading current in circuit containing the photoelectric cell.

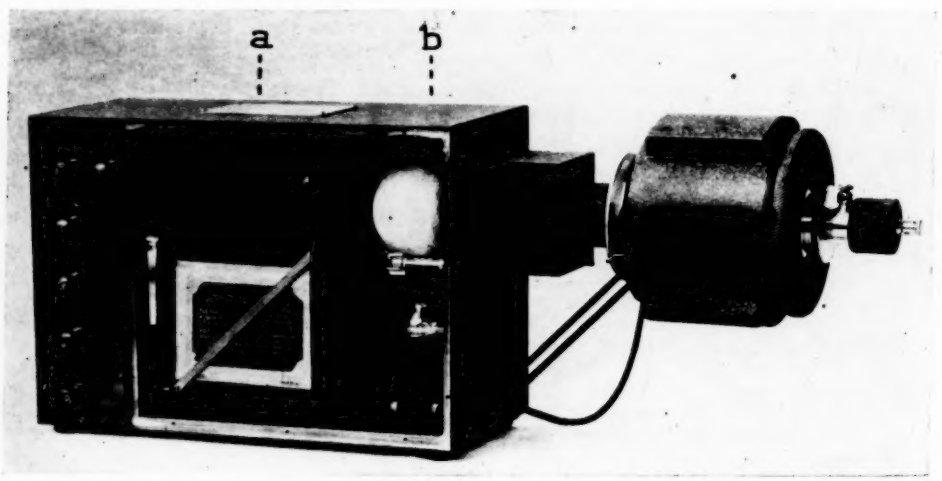

Fig. 2.—The photoelectrometer. *a*, Showing the one stage of amplification with vacuum tube and *b*, the photoelectric cell in position.

5. The "A" battery terminals are now connected to the corresponding binding posts on the instrument ("A +" and "A −") (Fig. 2).

6. The positive (+) terminal on the micro-ammeter is connected to the terminal on the instrument marked "M+" (Figs. 1 and 2).

The foregoing connections are all permanent and are left undisturbed when the instrument is turned off. The shutter is always left closed while connections are being made and the protective resistance controlled by the

lower knob on the front of the panel is turned to the position marked "in." Temporary connections are made only when the instrument is to be used and must always be made in the order described.

TEMPORARY CONNECTIONS

7. The terminals from the "A" battery are connected to the terminals on the lamp house (Figs. 1 and 2).

8. The filament rheostat (upper right hand knob) is turned "on" until the milliammeter on the end of the housing shows 250 milliamperes (0.25 amperes) (Fig. 1).

9. The positive (+) terminal of the "B" battery is now connected to the binding post on the instrument marked "B+." The micro-ammeter will now register 70 to 80 micro-amperes (Fig. 2).

10. The negative (–) terminal of the 1.5 volt "dry cell" is now connected to the binding post on the instrument marked "1.5 –" (Fig. 2).

11. The positive (+) terminal of the 1.5 volt "dry cell" is also connected to the negative (–) terminal of the 4.5 "C" battery. When this is done, the needle on the micro-ammeter will return nearly to zero (0).

12. The lower knob on the panel of the instrument which controls the protective resistance in series with the meter is now turned slowly toward the position marked "out," at the same time the position of the needle is carefully watched for any slight changes. If there is any change in the position of the needle, the meter is brought to a zero reading by slowly adjusting the upper left hand knob on the panel. The protective resistance should finally be turned all "out" (Fig. 1).

13. A clean container (standard spectroscopic absorption cell) is placed in the compartment in the instrument and the shutter is opened to admit light from the lamp house to the photoelectric cell. The deflection of the needle must indicate a current flow of 100 micro-amperes and the iris diaphragm of the shutter must be adjusted until the needle shows exactly this deflection.

The degree of deflection with an empty container must always be from zero (0) to 100. The shutter must always be closed immediately after a reading has been made.

To disconnect the instrument, operations *12*, *11*, *10*, *9*, *8*, and *7*, are performed in reverse order and in an opposite manner.

DILUTING THE BLOOD

No essential change has been made in the method of preparing the diluted laked blood as described in our first paper. Twenty cubic centimeters of the diluting fluid, 0.1 per cent sodium carbonate, is measured into a 50 c.c. centrifuge tube with a burette. For convenience twelve centrifuge tubes are placed in a block constructed for this purpose. It is necessary to use blood obtained by venipuncture, as it has not been found possible to measure accurately 0.1 c.c. of blood by using drops of blood from the ear. It is therefore most convenient to make the dilution at the same time that the patient is being bled for tests on the serology or chemistry of the blood. The blood may be pipetted from a small tube immediately after it has been placed

therein. We have continued to use the pipette constructed for the Kahn test. These pipettes are of 0.2 c.c. capacity and are graduated in thousandths of a cubic centimeter. The blood is drawn just above the 0.1 c.c. mark, and the excess blood is wiped thoroughly from the outside of the pipette with a damp towel. At the same time the column of blood is drawn down exactly to the 0.1 c.c. mark by placing the towel against the tip of the pipette. It is then blown into the 20 c.c. of carbonate solution. The diluent is drawn up into the pipette three or four times and expelled into the tube until the blood is washed from the pipette. In doing this care must be taken to do all blow-

THE DETERMINATION OF HEMOGLOBIN WITH THE PHOTOELECTROMETER

MICRO-AMMETER READING	HEMOGLOBIN, GM. FOR EACH 100 C.C.	MICRO-AMMETER READING	HEMOGLOBIN, GM. FOR EACH 100 C.C.
30.0	23.58	55.5	11.20
30.5	23.10	56.0	11.05
31.0	22.75	56.5	10.85
31.5	22.5	57.0	10.70
32.0	22.15	57.5	10.60
32.5	21.80	58.0	10.40
33.0	21.50	58.5	10.25
33.5	21.25	59.0	10.10
34.0	20.85	59.5	9.90
34.5	20.65	60.0	9.75
35.0	20.30	60.5	9.65
35.5	20.00	61.0	9.50
36.0	19.65	61.5	9.35
36.5	19.40	62.0	9.20
37.0	19.20	62.5	9.00
37.5	18.90	63.0	8.85
38.0	18.65	63.5	8.70
38.5	18.40	64.0	8.55
39.0	18.15	64.5	8.40
39.5	17.85	65.0	8.25
40.0	17.60	65.5	8.10
40.5	17.40	66.0	8.00
41.0	17.15	66.5	7.85
41.5	16.90	67.0	7.70
42.0	16.70	67.5	7.55
42.5	16.50	68.0	7.40
43.0	16.25	68.5	7.30
43.5	16.00	69.0	7.18
44.0	15.80	69.5	7.00
44.5	15.60	70.0	6.88
45.0	15.40	70.5	6.75
45.5	15.20	71.0	6.67
46.0	14.95	71.5	6.48
46.5	14.75	72.0	6.35
47.0	14.50	72.5	6.20
47.5	14.30	73.0	6.05
48.0	14.10	73.5	5.90
48.5	13.90	74.0	5.80
49.0	13.70	74.5	5.70
49.5	13.50	75.0	5.55
50.0	13.35	75.5	5.40
50.5	13.15	76.0	5.25
51.0	12.90	76.5	5.15
51.5	12.75	77.0	5.00
52.0	12.55	77.5	4.85
52.5	12.35	78.0	4.75
53.0	12.20	78.5	4.60
53.5	12.00	79.0	4.45
54.0	11.80	79.5	4.35
54.5	11.60	80.0	4.20
55.0	11.40		

ing with the tip held above the solution in the centrifuge tube. In other words, the exhaled breath must not be "bubbled" through the dilution fluid, as the carbon dioxide of the breath may neutralize the alkalinity of the solution sufficiently to cause slight precipitation of globulin and thus cloudiness of the solution. The tube is now thoroughly shaken with a rotary motion to facilitate complete dilution. Although this minute description of this part of the technic may sound trivial, it is exceedingly important that all dilutions be made in a standard manner. Check dilutions may be made until one is sure of the technic. Usually about four dilutions can be thus prepared from one sample of blood by working quickly, as one dilution can easily be made in less than a minute.

THE METHOD OF MAKING A READING

The spectroscopic absorption cell should be thoroughly cleaned with water, alcohol, and ether. The empty container is first placed in the photoelectrometer and the shutter opened. The light should be so adjusted by means of the iris diaphragm that the swing of the ammeter is exactly from 0 to 100. The shutter is then closed; the cell is filled with diluted blood and again placed in the apparatus. On opening the shutter, the swing of the ammeter will be less than from 0 to 100, depending on the density of the solution of hemoglobin. As one of the oxyhemoglobin bands in the spectrum lies in the same region as that represented by the light transmitted through the filter, the increased density is indicated at once by a decrease in current flowing to the ammeter. The reading on the ammeter is readily translated into grams of hemoglobin in each 100 c.c. by referring either to the curve (Fig. 3) or to the tabulation.

In calibrating the instrument we have made hundreds of determinations ranging from the very low readings of the severe types of anemia to the very high quantities found in polycythemia. We have also demonstrated that the time necessary for making a determination need not be more than two minutes. In fact we know of no other accurate procedure in hematology that can be performed as quickly.

In a series of experiments made to determine any fading due to standing any length of time, it was found that after two or three hours' standing there was less than 3 per cent loss in color. This fading occurs in the first two or three hours and apparently there is no further loss, as shown in readings made four to eight hours later. Readings, therefore, should be made as soon as possible after the dilution is made.

It is not in the province of this paper to discuss problems in hematology, nor to attempt to establish normal standards for the hemoglobin content of human blood. Many clinicians may find it hard to break away from an established custom of thinking of hemoglobin in terms of "per cent," based on some arbitrary figure taken as 100 per cent. In such time-honored calculations as that of the so-called color index, it may appear difficult to accommodate the new figure to the old method of making a ratio between hemoglobin and erythrocyte count percentage. However, if as a compromise figure for normal hemoglobin, 16.66 gm. is taken as 100 per cent, and 5,000,000

is taken as 100 per cent for the erythrocyte count, then the color index is easily estimated by the following simple formula:

$$\frac{\text{Gm. Hb. in 100 c.c.} \times 6}{\text{RBC count in millions} \times 20} = \text{Color index, normally 1.}$$

For example, if the number of grams of hemoglobin equals 15 in 100 c.c. and the erythrocyte count is 4,500,000 then

$$\frac{15 \times 6}{4.5 \times 20} = \frac{90}{90} = 1.$$

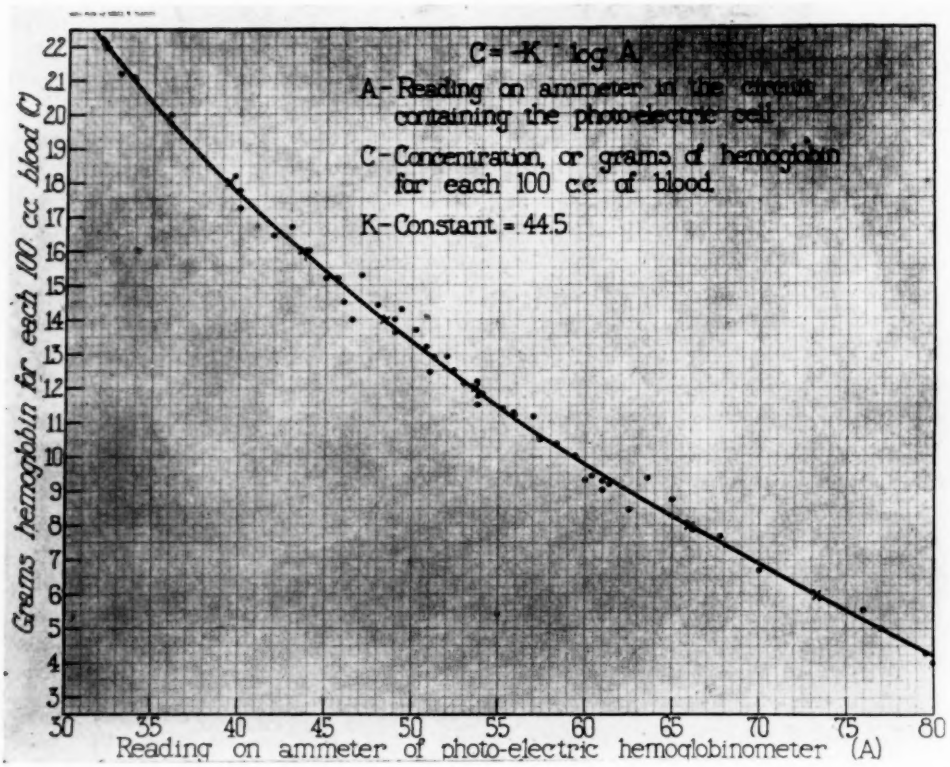
C = K log A
A - Reading on ammeter in the circuit containing the photo-electric cell
C - Concentration, or grams of hemoglobin for each 100 c.c. of blood
K - Constant = 44.5
Grams hemoglobin for each 100 cc blood (C)
Reading on ammeter of photo-electric hemoglobinometer (A)

Fig. 3.—The relationship between the readings on the photoelectrometer and the grams of hemoglobin determined by the van Slyke method.

We realize that the figure given is arbitrary and does not conform to any standard that has been established; on the other hand, it must be remembered that there is a wide variation in figures, both above and below that given, which have been reported as the normal average. It is not our purpose to discuss at this time the subject of the correct normal hemoglobin value, but merely to suggest a solution to the question as to how one may determine the color index.

In conclusion, we wish to state that we believe that the modified instrument as described and demonstrated at this meeting can be used satisfactorily for routine work, especially if a large number of determinations must be

made daily. We also call attention to the fact that the photoelectrometer may be adapted to many other uses in colorimetry. We shall prepare calibrations for the more common tests of the chemistry of the blood as soon as possible.

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DISCUSSION

Dr. Walter M. Simpson.—In comparing this with the methods in ordinary use, such as the Dare, the Sahli, etc., what percentage of error is there in those methods compared with the findings with the photoelectric hemoglobinometer? How do your findings check with the van Slyke and Newcomer determinations.

Dr. A. H. Sanford (closing).—It is a very important question. Perhaps we should have gone back to last year's paper. The curves were made and checked with the van Slyke oxygen capacity method. That is considered the standard method. Frankly, I believe this photoelectric instrument is a better method so far as accuracy is concerned than the Newcomer method and checks within 5 per cent. Several hundred colorimetric determinations, one right after the other, are very tiring to the eyes. I know that this photoelectric method will relieve the technician a great deal in routine work, just in that one respect of relieving eye strain. Technicians also differ in the way they match colors. We hope the photoelectric "eye" will stand up and not get fatigued.

RHAMY TRIPLE STAIN FOR FROZEN SECTIONS*

By B. W. Rhamy, M.D., Ft. Wayne, Indiana

FROZEN section tissue diagnosis in the operating room has become so deservedly popular that improvement in the technic should meet with favor. In my hands the greatest drawback was a satisfactory stain. Unna's polychrome methylene blue worked if one was fortunate enough to succeed in making a good batch, but it always seemed that when the time came for using it, it was deteriorated. Besides a permanent mount could not be made. Terry has improved this stain wonderfully but his stain also has the drawbacks, that it is difficult to make, fades quickly, cannot be used on all tissues, and tissue stained with it cannot be mounted permanently. Turley has suggested a quick hematoxylin-eosin stain, which however has many steps, and, in my hands failed to produce satisfactory stains in the time given, due to the fact that unfixed tissues do not take stain as readily as do fixed tissues. Of course frozen section tissue diagnosis must be done quickly, the staining method must differentiate the cells and their arrangement in the tissue so that their pathology may be quickly recognized.

I offer the following staining method as one that I believe fulfills these essentials and having the additional advantage that the section examined can be mounted permanently and that it does not fade.

STOCK SOLUTIONS

Solution I.—Saturated alcoholic solution basic fuchsin for bacilli (Grubler).

Solution II.—Saturated aqueous solution eosin.

Solution III.—Saturated alcoholic solution methylene blue for bacteria.

Solution IV.—Thirty per cent grain alcohol.

PREPARATION OF STAIN

Take of	Solution I	3 to 5 c.c.
	Solution II	5 c.c.
	Solution III	15 c.c.
	Solution IV	q.s. to 100 c.c.

The color of this mixture should be purplish blue. The fresh stain can be used at once but is better after ripening for forty-eight hours. Should the color be blue or reddish blue, due to atmospheric conditions or to poor quality of dry stains, let stand to ripen for a week or two. When stain is right, B. coli stains violet while B. typhosus and B. paratyphosus stain pink, B. diphtheriae rods and bars stain blue with pink interspaces.

*Demonstrated before the American Society of Clinical Pathologists. Portland, Oregon, July 5, 6 and 8, 1929.

METHODS FOR FROZEN SECTIONS

1. *Wet method:* Cut tissue into 4 per cent formalin. Float section on slide, cover with stain, and count 40. Rinse with water or dextrose solution and examine.

2. *Permanent mount (time sixty seconds):*

1. Cut tissue into 4 per cent formalin solution.

2. Section can be floated on slide and stained or can be lifted with a needle or bent glass rod and dipped in the solutions.

3. Cover with stain ten or fifteen seconds (count 30 or 40).

4. Wash off excess stain with water.

5. Ninety-six per cent alcohol (or denatured) until excess stain is removed (five to ten seconds).

6. Absolute alcohol three to five seconds.

7. Xylol three to five seconds.

8. Mount in neutral xylol-balsam.

3. *For Terry's razor sections:* This stain can be substituted for Terry's stain. The stained picture is practically the same by both stains as shown by simultaneous staining on the same tissue by Dr. Terry and myself at the meeting of the American Society of Clinical Pathologists, Portland, Oregon, July 5 to 8, 1929. The difference between the stains was that my stain takes about five seconds longer to produce the same depth of stain. The simplicity of preparation of my stain, its keeping qualities, and the fact that permanent mounts can be made with it are its advantages.

QUICK METHOD FOR MOUNTED SECTIONS (TWENTY-FOUR HOURS)

1. Five to 10 per cent formalin one hour.

2. Acetone two changes one-half to two hours each depending on size of the block of tissue.

3. Acetone, absolute alcohol, ether, equal parts one-half to two hours.

4. Absolute alcohol and ether, equal parts one-half to two hours.

5. Thin celloidon one-half to two hours.

6. Thick celloidon, overnight.

7. Mount and cut.

8. Lift section with needle and immerse in stain (count 30).

9. Wash in water.

10. Ninety-six per cent alcohol (or denatured) to differentiate or until stain ceases to come out (about ten seconds).

11. Absolute alcohol.

12. Xylol.

13. Mount in neutral xylol balsam.

This stain can be used on any sections of hardened tissue, whether frozen, mounted in celloidon or paraffin. It is an excellent counter stain for tubercle bacilli in tissues.

STAINING PROPERTIES

This stain tends to differentiate itself after mounting. Sections can be left in alcohol a reasonable length of time, but can be decolorized if left unduly long or by prolonged exposure to direct sunlight.

Epithelial cells take a light blue stain, endothelial cells lavender and small round cells deep blue. Glandular epithelial cells take on a sky blue, the nuclei of spindle cells stain a lavender blue. Connective tissue fibers stain pink while muscle fiber takes rose red to magenta. Mast cells show dark blue with dense blue granules, erythrocytes stain pink to orange, lymphocytes deep blue with magenta cytoplasm. Polynuclear cells show blue nuclei with pink cytoplasm. Concretions and mucus stain blue while caseous material stains pale red. Degenerated areas stain light blue to pink. Spermatozoa stain with the head blue, connecting piece dark red, and tail pink.

While this stain is intended for tissue it is useful as a triple stain for blood smears and for bacteria.

For Blood Smears (Quick Stain):

Better than eosin-hematoxylin and about as good as Wright's stain.

1. Fix blood smear with denatured or other alcohol.
2. Wash or blot.
3. Cover with stain one minute.
4. Wash with tap water, blot and dry.

Nuclei blue, neutrophile cytoplasm pink. Lymphocyte cytoplasm red to reddish blue. Basophiles same as in Wright stain, eosinophile granules pink to opaque white (like crystals). Red cells pink to orange, polychromatophilic cells red.

As a Stain for Bacteria in Smears and Cultures:

1. Fix with heat.
2. Cover with stain one half to one minute.
3. Wash and dry.

Negri bodies stain magenta with blue granules, nerve cells light blue. B. typhosus and B. paratyphosus A and B stain pink. B. coli and B. dysentery stain light to dark lilac. B. diphtheriae, beads and bars stain blue and interspaces pink. B. influenza and leptothrix, bars and polar bodies lilac, interspaces colorless. Pneumococci blue, with capsules in sputum glistening white in a red field. Staphylococci and streptococci blue (some streptococci stain pink). Gonococci deep blue (stand out in vaginal smears). Meningococci (in cultures) live cocci lavender, old cocci pink. Mic. catarrhalis (in cultures) live cocci blue, old cocci pink. Blastomyces (yeasts) blue, capsules pink. Spirochetes, some pink, some lavender. Mould, mycelium dark red or lavender, spores blue, capsules colorless. Spores white beads with violet capsules.

I have tissue, blood and bacterial specimen slides which were stained by these methods in 1927 which are still well stained. Some of these slides were shown at Portland last year. In making this stain be sure to have reliable dyes. My experience with some makes was disappointing.

THE EFFECT OF BILE ON THE AGGLUTINATION REACTION*

By Ruth Gilbert, A.M., M.D., and Marion B. Coleman, B.S.,
Albany, New York

Of the factors that may lead to irregularities in agglutination tests for typhoid and paratyphoid fever, variations in the cultures used are probably the most important. Besides the differences in agglutinability of various strains of typhoid and paratyphoid bacilli, cultures maintained in broth or on agar, which have been satisfactory for weeks or months, may suddenly show spontaneous clumping, loss of motility, or other undesirable qualities. The bacteriolytic property of certain sera, if not rendered inactive, may also furnish a source of error. In a study of means whereby this property might be inhibited, it was found that heating at 55° C. for one-half hour,[1] while otherwise satisfactory, at times lowered the agglutination titer. Sodium citrate likewise proved effective, but a concentration sufficient to inactivate completely the lytic properties is hypertonic. Ryti[2] has reported that this reagent eliminates the so-called "zone of inhibition" in sera. Of the various methods studied, the use of bile in the culture medium or as a diluent of the sera proved the most satisfactory, since it was found not only to inactivate the lytic properties of the serum, but also to have a favorable action on cultures grown in medium containing it.

It is generally known that the growth of B. typhosus may be stimulated by the presence of bile in culture media, especially in those used for isolating the organisms from blood. Hauduroy,[3] Caublot,[4] and De Jong and Hauduroy[5] attribute this to a destruction of the lytic activity of the bacteriophage. Our results can hardly be explained by this theory, however, since the presence of bile has inhibited the lysis of killed as well as living organisms. Pfannenstiel,[6] and Pfannenstiel and Kortman[7] have shown that the presence of bile or bile salts renders inactive the bactericidal, bacteriolytic, and other complementary properties of sera. Formicola[8] reports that bile accelerates the agglutination reaction and may increase the titer of serum markedly.

During two years, our staff members have performed in duplicate 350 microscopic tests using typhoid bacilli grown for eighteen hours at 37° C. in extract broth and in peptone-water medium containing 3 per cent fresh ox bile, the optimum amount indicated by preliminary tests. The stock cultures were kept on infusion agar.

Preparation of Bile Medium.—Combine 1 kg. distilled water, 10 gm. peptone, and 30 gm. fresh (5 gm. dehydrated)* ox bile, and boil vigorously for from three to five minutes. Leave in a cold room overnight and filter

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From the Division of Laboratories and Research, New York State Department of Health, Albany, New York.

*Peptone water containing 0.5 per cent dehydrated bile has been used with equally good results providing it is freshly filtered. A precipitate forms more rapidly in this medium than in that containing fresh bile.

through paper until clear. Dispense in bulk and sterilize in steam at 121° C. for twenty minutes. Store in the cold room. Decant or filter through paper before tubing and sterilize in steam at 121° C. for twenty minutes. Since solutions containing bile develop precipitates, medium containing it should be used within a week after filtration.

The specimens examined included dried blood and sera. The tests were made by the hanging-drop method and incubated one hour at 37° C. Usually the work was done by two individuals, and the results were compared after completion. No lytic activity was observed with the culture in medium containing bile, while lysis was frequently so marked with the one in broth that it was necessary to repeat the examination after inactivation of the serum. Otherwise, appreciable differences did not occur at any time in the reaction obtained with the two cultures. The usual fluctuation in motility and the variation in the character of the clumping in the broth culture were noted from time to time, but the culture in media containing bile was uniform.

Cultures grown in bile medium have not been found entirely satisfactory for macroscopic agglutination tests, the lytic properties of some sera remaining active when tested with this culture. However, excellent results are obtained with sera diluted with 0.85 per cent salt solution containing 3 per cent fresh ox bile and allowed to stand for a short time before the addition of either a living broth culture or a formalinized suspension in 0.85 per cent salt solution.

SUMMARY AND CONCLUSIONS

When cultures of B. typhosus or B. paratyphosus have been grown in media containing appropriate amounts of bile, no evidence of loss of motility, spontaneous agglutination, or lysis has been noted in microscopic agglutination tests. Also, the addition of similar amounts of bile to the salt solution used in diluting sera for the macroscopic agglutination test has rendered their lytic properties inactive. The action of the bile may be due to a lowering of the surface tension, but further study is needed to determine the basis for the reaction.

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GINGIVITIS*

V. The Character of the Exudate in Gingivitis

By Robert A. Keilty, M.D., Washington, D. C.

THIS is the fifth in a series of papers on the general subject of gingivitis, and it is presented from the bacteriologic viewpoint. My approach to the subject will be found in previous reports.[1, 2, 3, 4] While the problem as a whole rightfully and strictly belongs to the dentist[4] this phase of the report may interest those working in mouth bacteriology.

As one studies the bacteria of the mouth the maze of possibilities becomes endless. It is far from the simple isolation of a streptococcus, a staphylococcus, or a diphtheria bacillus as outlined in the textbooks. Where one delves into the relationship of the bacteria found in thousands of mouths and attempts to check the findings against the pictures of gross pathologic conditions, as viewed from the conceptions of the pathologist, the problem at times seems hopeless.

By gingivitis I mean any inflammatory process involving the soft tissues about the necks of teeth. Where the lesion has extended to the bone, the term gingivitis may be augmented by periodontitis. As far as the exudate is concerned all factors are included under gingivitis. I am not attempting to discuss causal relationship here, and I am mindful of all the other factors which may be present in a given case. These have been discussed in other papers. It would seem wise to group all forms of inflammatory changes in the gums from acute trench mouth and Vincent's angina to pyorrhea under the general terms of gingivitis and stomatitis, qualified by their appropriate descriptive term.[2] This is especially true the more one studies the bacteriology and tends to simplify the subject as a whole right in the beginning.

In the first place, while the critical study of a thousand or five thousand mouths will place them in groups subject to classification, after all, each individual mouth is an entity and the factors found must be evaluated per se as far as that particular mouth is concerned. Great individual variation occurs.

The perfectly normal mouth is a rare condition. The gingivae are involved to some extent in over 90 per cent of hospital admissions and even higher in general dental practice. Normal gums[2] are firm and pale, abnormal gums are red and inflammatory in appearance. Normal gingival sulci are tight and contain nothing but a few desquamated cells, an occasional leucocyte and very few bacteria, if any, on smear. The presence of actual material about the necks of teeth and in the gingival sulci constitutes an exudate. Many mouths, where cleansing is not a routine after eating, contain foodstuffs about the necks of teeth but this is easily differentiated from exudate. The exudate is, of course, the product of reaction from the subgingival and periodontal

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tissues, whatever the cause may be. Bacteria and protozoa are present in abundance in this exudate. While bacteria are present in culture from the necks of teeth and gingival sulci of perfectly normal mouths, they are not present in abundance. They are likely to be more nearly single organisms, very often a green hemolytic streptococcus and not the heavy mixed flora found in the definite exudates.

An exudate is the product of reaction on the part of the tissues and blood vessels involved with the causal bacteria. It may be serous, serofibrinous, or suppurative. It may appear in the tissues with rubor, calor, dolor and tumor or it may appear on the surface with serum, false membrane or pus. This is exactly what happens in the soft structures about the teeth and as such a gingivitis may be acute, subacute or chronic in type with exudate in the sulci or on the surface, membranous, ulcerative or suppurative in character.

In the examination of an exudate all technical means at our disposal should be used, fresh smears, stained smears, dark-field smears and cultural methods, aerobic and anaerobic. In the first part of my work I relied on smears for diagnosis, but now I would suggest every means possible until all the tangles of technic are straightened out. Much can be learned from both smears and cultures and these methods have been previously described.

Regardless of whether the gingival condition is acute or chronic certain products of exudate, bacteria and protozoa, are common to all conditions. The more acute the process is, the more intense the inflammatory picture will be with congestion, hemorrhage, false membrane and ulceration. The more chronic the condition, the more the picture resembles passive congestion with suppuration.

Red blood cells are difficult to keep out of smears because most of the gingivae involved bleed at the slightest touch. It is well to avoid hemorrhage as much as possible in making smears. Red blood cells appear as a part of the exudate but in their escape they are promptly attacked by the bacteria especially the active spirilliform organisms. It is not uncommon to see a single spiral bacterium shake a red blood cell in violent action and to see two or three attached and working on a cell so that in the movement it may be easily mistaken for a trichomonas. At times a red blood cell is literally surrounded by spirilliform organisms which seem to be deriving their nourishment by the attachment of their head ends, if such they be.

White blood cells are always present in every exudate. They may appear as scattered cells in the mild and treated cases, in the acute and chronic cases they appear in clumps and dense masses enmeshing islands of active bacteria. Many excellent examples of phagocytosis are to be seen in stained specimens. Bacteria do not actively attack the pus cells as they do the red blood cells but, now and then, a pus cell is evidently in a chemical state agreeable to the bacteria and they present a mass attack similar to that seen against the red blood cell.

Epithelial cells are nearly always present. In the acute ulcerated cases they appear as layered masses, while in the treated cases and where repair is active, excessive epithelial groups may be taken as an indication that repair

is progressing. In the chronic cases, individual epithelial and connective tissue cells are scattered about as single cells.

The débris of food cells, vegetable, meat fiber, oil droplets as in the examination of feces are easily recognized in fresh smears after a little experience. These are found where cleansing is inadequate, in the tight interproximal spaces and sometimes in the deep pockets of chronic cases. They are to be recognized and have value in the diagnosis only as such.

Calcareous material, the subject of much dental expression, about the necks of teeth as salivary calculus and deep in gingival sulci as serumal calculus, is the calcareous infiltration of necrotic material as expressed elsewhere in the body, particularly favorable in this position. It is easily recognized grossly as a brittle, usually slightly yellow stained, calcareous material more or less adherent to the teeth. Microscopically, it is amorphous or partly spicular and when dissolved by acid in a slide preparation leaves a protoplasmic débris with bacteria. The whys and wherefores of this calcareous deposit is a subject discussed for years, but the mechanism of which I do not think has yet been satisfactorily cleared up. At times there are other crystalline deposits such as cholesterin but they are not the rule.

The bacteria present in gingival exudates is the most important element, the most perplexing and the most in need of systematic study.

The spirilliform group stands out first and foremost. Every pathologist is asked at times to make a smear for "Vincent's," and if he is careful every time he makes such a smear, he finds the combination of spirilla and fusiform bacilli and makes a positive diagnosis. What does it mean? Nothing, unless one is familiar with the conditions present. The atmosphere and background on the spirilla in the mouth is very hazy. Most of the textbooks describe Vincent's angina and say that it is due to a spirilliform organism with a fusiform bacillus, having certain staining characters. They describe the attempts at cultivation and a different nomenclature is found in every book.

The work of Tunnicliff began to clarify the situation but has not yet brought it to a successful conclusion. Noguchi probably knew more about spirochetal forms than anyone else, but as far as the mouth is concerned he also left us at the halfway mark. In the *Manual of Determinative Bacteriology*[5] the spirilla of the mouth are now classified under Order VI, Spirochetales, Family 1, Spirochetaceae, Genus IV, Borrelia and Genus V, Treponema. Under Genus IV, No. 12, Borrelia vincenti is briefly described "spirals: 0.3 by 12 to 25 microns. Habitat: The oral cavity. Occurs in association with the so-called 'fusiform' bacillus in Vincent's angina and allied infections." Under Genus V, to which Treponema pallidum belongs there are three types of mouth spirilla which Noguchi described: No. 3, Treponema microdentium, Noguchi; No. 4, Treponema macrodentium, Noguchi; No. 5, Treponema mucosum, Noguchi.

Until such time as a definite and satisfactory method for the isolation and cultivation of mouth spirilla is accomplished, this may come at any moment, it would simplify matters if all spirilliform organisms in the mouth other than T. pallidum whether they be long or short, active or motile, were included under the general grouping of Borrelia vincenti. All other references for the

time might be discarded. At the same time the importance of maintaining the necessity for a symbiotic relationship with fusiform bacilli could be discarded. Let us consider B. fusiformis and spirilla as definite entities until they are more satisfactorily studied. As far as the spirilliform organisms are concerned we are now at the stage the streptococcus was in several years ago when it was spoken of as S. longior, S. brevior. These were mere terms and meant that their terminology represented the state of our knowledge at that time.

Borrelia vincenti appear constantly in gingival exudates. They are best seen in fresh saline smears under the high dry powers of the microscope. As a rule the more active the infection is the longer the forms are, the more active they are and the more spirals they have as compared with the looser more undulating types seen in chronic cases. In these acute cases at times they closely resemble T. pallidum under the dark-field and while they can as a rule be differentiated, occasionally this is impossible. For those who are not perfectly familiar with T. pallidum under the dark-field a positive diagnosis in acute suspected syphilitic lesions of the mouth should be fairly certainly made before one is too dogmatic on the differentiation.

Considering all spirilla not definitely Treponema pallidum found in the mouth under the generic heading of Borrelia vincenti, they are subject to description. Examined in fresh saline suspension, they vary from 3 to 15 microns in length and are uniformly thin with slightly pointed ends. Usually approximately the same size holds for the predominant type present in a given mouth. As a rule the more chronic the case is the shorter the prevailing forms are and the more acute the case the longer the forms are. In the same way the more inactive the process is the more sluggish the organisms are in movement and the more acute the case the more active. In activity they have a characteristic movement which is sharp and jerky with an instant period of complete quiet. They may move in a very small sphere backward and forward, or they dart in all directions. They are gram-negative as a group, and when gram-positive forms are seen, something is usually wrong with the stain. They take several stains including fuchsin and gentian violet. In stained preparations they are more drawn out with an undulating wave and are, therefore, correspondingly longer. I have seen at times a wavy membrane but this is not the rule. At times in the dark-field they seem to have either a splitting longitudinally or actual flagella but this also is not the rule. Their bodies are uniformly stained and intracellular granules is not the rule.

Many attempts at cultivation have been made but nobody has described entirely satisfactorily a method which will allow definite pure culture studies. I have tried many possibilities. At times anaerobically I have thought I had more than a transfer but they always die out. Recently I have had some actual growth on twenty-four-hour blood agar plates in apparent symbiosis with staphylococcal colonies. I have thought at times that fusiform bacilli were showing evidence of transmutation to spirilla but I have never had any actual success. I know of several studies now being carried out along anaerobic lines for the cultivation of spirilla and any one of these may prove

successful any day. Unless spirilla can be made to start under aerobic conditions they must be started in mixed culture first and eventually, by transfer, made to grow at the expense of their symbiotic fellows and not otherwise as now happens.

In the gingival exudates, spirilla classed under the genus Borrelia can be easily detected by fresh, wet smear or by stained smear and their presence indicates inflammatory action. They are not present in normal mouths and they disappear in cases appropriately treated. They are transferred, of course, from mouth to mouth mainly by kissing. At the present time there is a marked increase in cases of acute ulcerative gingivo-stomatitis (trench mouth), due in part to great numbers of active borrelia which probably represent virulent strains of the organism.

The next most common organism in gingival exudates is the fusiform bacillus. This is also loosely described in textbooks but in *Determinative Bacteriology*[5] is under Order II, Actinomycetaceae, Family II, Myobacteriaceae, Genus III, Fusiformis, No. 2 Fusiformis dentium, Hoelling. It is ten to twelve microns long, slightly curved with pointed ends. It is usually not motile but at times it is quite motile, swimming about in a slow graceful fashion. It is gram-negative. While it may be obtained both on blood agar plates and in anaerobic cultures or by a combination of both, it is difficult to obtain in pure culture. It is readily recognized in wet and stained smears, varies in numbers and while it is usually present in the same smears with borrelia it may appear independently. It is not present in perfectly normal mouths.

The next organism in order of frequency is a small comma-like bacterium more like a tiny polywog with a small body and a tail, not an actual flagellum. This has the greatest motility of any of the motile forms and darts all over the field. It is apparently described under a variety of names and again until more definitely placed, Vibrio sputigenus (M.D.B.[5]) best places it.

There is a more or less constant longer broader bacillary body with blunt or even square ends, nonmotile, sometimes in pairs or short chains and always gram-positive. This belongs to the lactobacillus group. It has recently been described as an associated cause of dental caries but it is present in most exudates without any evidence of caries.

Under the more or less general term of leptothrix, mycelia are present in most exudates but more in the sordes of neglected mouths and in lessened numbers in the more acute and advanced cases of gingivitis. These appear as long interwoven mycelial threads, are nonmotile and as a rule gram-positive. Occasionally branching is seen but this is not the rule. From cultures in symbiosis with the coccal colonies they appear as long swollen threads with many intracellular bodies. They are the least pathogenic of all the various types since they appear in abundance in the least active mouths.

In many exudates there are found clumps of bacilli, of the gracefully moving fusiform type, which are in three formations. First they are occasionally definitely agglutinated, much more often they appear as developing rosettes. There is a central body mass and from the periphery the bacillary bodies protrude as in a ray fungus. In the third form the bacillary bodies

are directed and folded in one direction so that the appearance is that of the tuft of a pineapple. Similar rosettes were shown to me by Broders last year in some tonsil sections. I am not sure where they belong but they are a frequent finding in gingival exudates.

There are occasionally other forms of fungi, myceelia and yeasts. They are inhabitants of individual mouths and do not belong to any group constantly found in exudates. The yeasts which have been suspected by some writers are not present in numbers of cases but occasionally a single case will show them in abundance.

The other bacteria present in exudates can best be determined by cultural methods and the blood agar plate brings out most of them. The flora is more or less constant for a given mouth, shows the same type from individual sulci and pockets with variations of predominant colonies from different locations. The exudates are influenced by seasonal changes, heavier in winter and lighter in summer. For example the pneumococcus, predominant in winter, almost disappears during the summer. Gingival floras bloom in types at epidemic times during the winter and keep pace with the general tonsil and pharyngeal floras. There are forty-six different varieties of bacteria described in the *Manual of Determinative Bacteriology*[5] as having been isolated from the mucous membrane of the mouth or from the sputum. I believe I have isolated thirty-three of these different types from gingival exudates in this work so far and I know this will increase as the cultural studies are augmented and elaborated.

Different forms of staphylococcus and streptococcus are present in all exudates. The characteristic staphylococcus is a muddy, moist, glistening, mushroom, jelly-like colony in which other forms of white cocci grow and in which fusiform, bacillary, myceelial and vibrio symbiosis appear in the colony. This is especially true in the heavy parts of the smear and at points where it is confluent. The pure white and yellow staphylococcus is not the rule but at times predominates. At other times deep yellow and pink colonies appear in the older plates. The muddy staphylococcus is usually hemolytic, at times markedly so and at other times shows an opaque sheen in the zone of the hemolysis. The drier muddy colonies with gram-negative cocci are those of Neisseria catarrhalis (Micrococcus catarrhalis). The staphylococcus and the catarrhalis are closely allied and often only in the gram stain can they be differentiated, those cocci which are positive being staphylococci and those negative catarrhalis. Many of these colonies with gross variations distinguishable only under the dissecting microscope show marked variations in the cocci when stained. They appear as globoid bodies often with variations in size, masses, tetrads, diplococci and continue to transplant in their form or change markedly from plate to plate. There are characteristic muddy colonies of the general type of staphylococcus in all exudates which represent different strains in different mouths.

The streptococcus is subject to an even wider variation. The colonies are flat, moist, gray, pearly white, opaque and digesting. The hemolysis is small, marked, sharply defined, and green. The virid hemolysis predominates in most of the cultures. Streptococcal groups may be designated by their blood agar plate reaction and I am now working on their serologic grouping.

There are many other organisms isolated from the exudates of particular cases. In a recent unselected group of one hundred cases[4] showing all types of gingivitis, in addition to the different strains of the streptococcic and staphylococcic groups which make up the vast preponderance, the following organisms were found, pneumococcus, B. diphtheria, B. hoffmannii yeast and an unidentified bacillus. At times I have found B. coli, B. influenza but never B. typhosus nor B. tuberculosis.

The protozoa in the mouth form a very important link in the etiology of gingivitis. *The generally accepted viewpoint that they are harmless parasites is erroneous.* They apparently do not produce metastatic lesions outside of the gingivae but in the gingival sulcus they are most active irritants in the continuation of suppurative processes. It is not my purpose to review the literature nor the controversy here but merely to state their findings.

The most frequently occurring protozoa is Endameba gingivalis (Gros). This organism is found in 71 per cent of my cases. It appears most frequently in the deep pockets of the more chronic cases but it also may be found in acute cases. It is present in association with the other flora. It is not by any means the only etiologic factor; it is not "a specific cause of pyorrhea" but it is an important factor. It may in a very small group, less than one-half of 1 per cent, be the one factor which will clear the case up and in those cases it is of prime importance.

Endameba gingivalis[1] has been well described by Smith, Bass and Johns, Kofoid and Swezy and others. I would like to add here and present evidence later, that in addition to the vegetative forms it occurs in cyst form in common with other endameba. I have no evidence yet that it invades the tissue of the gingivae or that it appears in the intestinal flora in these cases.

The next most common protozoa is the trichomonas. This was first described by Steinberg 1862 who named three distinct forms (Wenyon). Wenyon suggests the generic name Trichomonas elongata, the first proposed by Steinberg as against T. buccalis of Goodey and Wellings. On the other hand, in order to differentiate easily from T. vaginalis and T. hominis, T. buccalis (Steinberg) would seem appropriate. Lynch[7] has studied the organism extensively in cultures. I have found it several times very abundantly in anaerobic cultures, sometimes it is found after a week's incubation when it was not seen in the original smear. In a series of positive gingival cases in which the feces were studied T. hominis, identical with T. buccalis was found in two cases. I believe after more careful search in a greater number of cases this protozoa will be found more frequently in both the mouth and feces and the relationship between the two more definitely decided. Trichomonas in the mouth is as a rule much smaller than T. vaginalis and more nearly the size of T. hominis in the intestine. In my estimation this parasite occupies the same relative position as to pathogenicity in gingivitis as does the endameba.

There is another much smaller protozoa which I have frequently seen and only lately have been more carefully searching for. This is a small motile cell with either a single flagellum as in the species Bodo or it is a form of infusoria with an attachment process. This is of frequent occurrence and warrants more critical study.

I am sure I have seen one or two definite Giardia in gingival exudates. For the past year I have been closely on the lookout but have not encountered a case. It is evidently very rare. The same may be said of Cercomonas. In my early work I have mistaken Cercomonas for Trichomonas, since I have not seen a single instance of Cercomonas during the past year. There are no other protozoa, neither vegetative nor cysts with which I am familiar, that have been seen in the gingival exudates thus far studied. I have been looking for Blastocystis hominis but do not believe that it occurs in the mouth.

DISCUSSION

There is a general tendency, because of the conditions present and the consideration of bacteria and protozoa as inhabitants of normal mouths to disregard their importance in pathologic lesions and to look for other factors in etiology. The more one studies these bacteria the more important they become and with treatment directed at them the greater is the improvement in results. The exudate about the sulci of teeth is even more complicated than the fecal flora, but it can be worked out relatively satisfactorily so that individual cases may be treated more intelligently.

Since the pathologic changes in the gingivae and bone about the teeth, of an inflammatory character, have all the earmarks of an infectious nature, it seems reasonable to give the bacteria and protozoa found their rightful importance and consider them in a direct etiologic rôle.

The apparently widely separated conditions known as "trench mouth" and "pyorrhea" become closely related on the basis of the study of their etiologic organisms. For this reason I have grouped all inflammatory changes in the gingivae under the heading of gingivitis and have worked out for each case its etiologic flora.

In studying the exudate, its gross and microscopic appearance must be considered from a purely cellular exudative standpoint. In the mouth extraneous cytology introduced in foodstuffs must be differentiated. The protozoa, Endameba and Trichomonas, are more or less common with Cercomonas, Giardia, Balantidium and Bodo occasionally seen. The spirilla under the name of Borrelia vincenti, the fusiform bacillus, B. acidophilus, Vibrio sputigenus and mycelia are constant findings. The staphylococcus and streptococcus as groups are also constantly present. A large number of other organisms may appear in individual mouths such as the pneumococcus, B. coli, B. influenza and virulent types of B. diphtheriae. All of these organisms have a direct bearing as etiologic factors of the gingivitis per se. In addition, the invasion of the subgingival tissue acting as a portal of entry becomes one of the most important focal points of infection for the production of metastatic pathology.

CONCLUSIONS

Definite gingival exudates appear in the sulci about the necks of teeth which are subject to a bacteriologic study in relationship to the disease present in the gingivae.

Perfectly normal mouths do not have exudate about the necks of teeth, therefore, the presence of exudate about the necks of teeth is an indication of inflammatory change in the gingivae.

On the basis of the bacteriologic study of gingival exudates such diseases now known as trench mouth, Vincent's angina and pyorrhea become different manifestations of a single entity, gingivitis.

The prevailing organisms are Borrelia, B. fusiformis, Vibrio sputigenus, a bacterial flora, B. acidophilus and mycelia.

The prevailing protozoa are Endameba and Trichomonas.

The study of the bacteriology and cytology of gingival exudates places a nonspecific bacterial etiology for gingivitis of equal, if not of greater, importance than the other factors now considered predominant by the dental profession.

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DISCUSSION

Dr. M. M. Patton.—This question of mouth bacteria is an important one and something which needs further work both in the cultivation and isolation of the bacteria causing it. It is surprising to find in some mouths, where they have large ulcers, etc., that you will find very few spirilla. In other cases where they have small ulcers or single ulcers it is surprising how many more spirilla you will find. I was glad to hear Dr. Keilty say that at the present time he paid no attention to the number of bacteria present. I think it is rather important that he does not try to classify the bacteria. We find in mouths where there are a great number of bacteria and where they are extremely motile that these are the worst cases. The dentists in our town are cognizant of the fact that removing teeth promiscuously without first having smears taken from the teeth is not a good procedure. I have seen large ulcers and death from just the removal of teeth. Some men think that the spirilla of the mouth have something to do with certain forms of anemia, particularly infectious mononucleosis and allied types. It occurred to me that there might be some relationship of the bacteria of the mouth, particularly the spirilla, to chronic myocarditis and arteriosclerosis. In the work of Warthin, in which he stained the tissue of the heart in sclerosis of the heart muscle, he almost always found spirilla and classified them as Treponema pallidum. This view has not been entirely accepted by others, but it might be well to think of these treponemes as some other form of spirilla closely resembling pallida types.

Dr. Frederick H. Lamb.—I have wondered if others have thought as I have about expressing the volume of the infected area in figures. If the diameter of the average tooth is about one-fourth of an inch, the circumference of the tooth is about three-fourths of an inch; and if the depth of the infection is three-eighths to one-half inch, it is easy to figure that, if there are twenty infected teeth, the infected area is equal to a ribbon of infection approximately fifteen inches long, and from three-eighths to one-half inch in width. One need have no doubt then about the teeth being a prolific source of infection, since we have an enormous number of bacteria in potential contact with the blood stream.

In regard to the last speaker's reference to Dr. Warthin's work on spirochetes; I

can say with certainty that the organism which Dr. Warthin speaks of as being treponema pallidum is without any doubt that organism.

Dr. E. C. Rosenow.—The facts brought out by Dr. Keilty are unquestionably of the greatest importance. Infections of the gingivae as a source of general ill health and the source of grave systemic disease has scarcely been considered by physicians and dentists. His presentation leaves no doubt that gingivitis is an important source of such infections. Of the numerous kinds of microorganisms at hand, none is perhaps as significant as the streptococcus, at least as far as relation to systemic disease is concerned. Drs. Cook and Stafney working with me in the section on Dental Surgery at The Mayo Clinic have shown conclusively that the exudates in periodontitis contain streptococci of the viridans group like those isolated from the apices of pulpless teeth and which also tend to localize and produce lesions in animals similar in location to those at hand in the patient from whom the material is obtained, elective localization.

I hope that Dr. Keilty's presentation will do very much to awaken interest in this important and greatly neglected source of infection.

Dr. Frank W. Hartman.—I would like to ask if Dr. Keilty sees any constitutional disturbances in any of these cases, especially in the marked cases.

Dr. Robert A. Keilty (closing).—The point that Dr. Hartman has just raised, might be discussed by some of the California men. A group in California working on etiologic factors dropped off it seems to me just as their evidence was convincing. They gave infectious factors a secondary rôle and constitutional disturbances a primary rôle. As a primary rôle a constitutional disturbance plays an important but a minor part in this group of cases. Tuberculosis and diabetes are secondary to other more definite gingival factors.

In answer to Dr. Rosenow, I am glad to know that Dr. Cook has shown that these organisms do the same as those isolated from the deeper pathologic areas. In fact the avenue of infection or portal of entry is more commonly by the gingival route than it is through the root canals in my opinion. There is no reason whatever for extracting a tooth because it is just dead, especially when it does not show any periapical pathology. Many dead teeth with root canal fillings are sterile and will remain so for a number of years.

The last point, there are entirely too many deaths starting in the extraction of a single tooth and ending in cellulitis of the face and neck. These are preventable cases when a mouth has had preoperative specific treatment from the standpoint of the gingival infections present. Under these conditions any amount of surgery can be done at one time when the danger of secondary flare-ups is reduced almost to nothing.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

C. DIPHTHERIAE: Hydrolyzed Serum Agar for the Isolation of C. Diphtheriae, Thompson, L. J. Infect. Dis. 45: 163, 1929.

A serum agar has been prepared by treating Loeffler's blood serum, dehydrated, with sodium hydroxide and incorporating the noncoagulated product in agar. The medium promotes the growth of diphtheria organisms, and inhibits certain other organisms found in the throat. It is easily available, easily prepared, can be safely sterilized in the autoclave and offers the convenience of an agar medium for the plating and isolation of pure cultures of Corynebacterium diphtheriae.

The formula follows:

Loeffler's blood serum (Bacto dehydrated), 40 gm.; water (at 40° C.), 250 c.c.; mix well; sodium hydroxide (normal), 150 c.c.; place in incubator at 37° C. for forty-eight hours; neutralize with 5 per cent hydrochloric acid to P_H 7.0 using bromthymol blue; add 2.5 gm. sodium citrate; when dissolved, titrate to P_H 6.4 with 3 per cent citric acid; to this add, while hot, an equal amount of 3 per cent agar solution; put in tubes or flasks as needed and sterilize in the autoclave at 15 pounds pressure for fifteen minutes.

VERRUGA: Cultivation of Bartonella Bacilliformis, Battistini, T. S. Ann. Facul. de Med. Lima 28: No. 10, 243, 1927.

The author's technic for cultivating Bartonella is quite simple. A small drop of blood from the finger of the patient is withdrawn by means of a pipette into semisolid medium (serum agar, for example), the end is sealed in the flame and the whole placed in an incubator at 28° C. Colonies are visible in five to six days. The individual bodies are 0.6 x 0.2 microns to 1.6 x 0.5 microns; they are gram-negative, markedly motile at the junction of medium and water of condensation, but flagella have not been demonstrated. In old cultures spirillar forms are seen, from 7 to 30 microns in length. Growth will not take place in fluid media, only on solid or semisolid, and under aerobiosis. Cultures had no action on any of the 17 sugars tested. The reaction of the medium may be between P_H 6.8 and 8.2, but the optimum is 7.4 to 7.6. A temperature of 56° C. destroys the organisms in ten minutes, of 60° C. in five minutes; 1 per cent formol, tricresol, or lysol in ten minutes.

Whether in culture or blood they will not pass through Berkefeld filters V or N. In citrated blood they remain viable at 14° and 26° C. for three and one months respectively; in culture at laboratory temperature (22° to 25° C.) they survive for sixty days, even though no precaution is taken to prevent the medium drying.

STAIN: A Modification of Mayer's Hemalum, Sass, J. E. Stain Tech. 4: 127, 1929.

Dissolve 50 gm. of alum $Al_2(NH_4)_2.4SO_4$ in a liter of boiling water. Remove from the hot plate and add 1 gm. of hematoxylin (obtained from Coleman & Bell, National Aniline Co., or Mac Andrews and Forbes). Add 1 gm. $NaIO_3$, cool and filter. The stain should be filtered whenever a "metallic" scum is present. The solution is best when fresh, but its staining properties are retained for at least six months. The keeping qualities are now being tested.

The slide to be stained is transferred from water to the staining fluid. It is washed in distilled water, then in tap water (or 1/100 Na_2CO_3), and again in distilled water. Finally it is dehydrated and cleared as usual. An aqueous or alcoholic counterstain may be used. This is primarily an histologic stain.

MOSQUITO: An Improved Method of Mounting Mosquito Larvae, Gater, B. A. R. Bull. Entomol. Research **19:** 367, 1929.

The mounting medium used consists of (in percentage) water, 10.0; picked gum arabic, 8.0; chloral hydrate, 74.0; glucose syrup, 5.0; glacial acetic acid, 3.0.

The living larva is placed on a slide and all surplus water is removed, some of the medium is dropped on it, and then a cover glass. Clearing begins as soon as the larva is dead (and with most species is complete in two hours, but with highly pigmented species not until perhaps twenty-four hours). After mounting for permanence the preparations are set aside to dry, a process that in a moist atmosphere may take three weeks, before being ringed. "The advantages claimed for this method are simplicity of technic and efficiency of clearing."

DIPHTHERIAE: A New Suspending Medium for Intracutaneous Virulence Test, Stone, R. B., and Weigel, C. Am. J. Pub. Health **19:** 1133, 1929.

STONE-WEIGEL VIRULENCE TEST SUSPENDING MEDIUM (AGAR-PEPTONE-BROTH)

Oven dried agar	0.2%	0.2 gm.
Water to make		100 c.c.
Boil briskly to dissolve agar; then add:		
Peptone (Witte's)	4.0%	4.0 gm.
Sodium chloride (c.p.)	0.5%	0.5 gm.
Beef extract (Difco)	0.3%	0.3 gm.
Dextrose (Difco)	0.2%	0.2 gm.
Boil at this stage in double cooker.		
Adjust to P_H 7.0.		
Then add n/1 Na(OH)	0.7%	0.7 c.c.

Sterilize at 15 pounds pressure for thirty minutes, after tubing; 1 c.c. per tube. Cork tubes and seal with paraffine. Care must be taken to prevent evaporation of stored medium since a very slight concentration increases the percentage of agar sufficiently to solidify the fluid. Different lots of shredded agar vary in "setting" properties; so each new lot should be tested.

UNDULANT FEVER: The Diagnosis of Br. Abortus Infection in the Udder of the Cow, Torrey, J. P. Am. J. Pub. Health **19:** 360, 1929.

By the application of the Huddleson rapid agglutination test to the milk, practically all animals carrying Br. abortus in the udder can be detected. The first few streams from each quarter are drawn into clean test tubes or vials. The strippings should never be used. A composite sample is of little value since only one quarter may be infected. About 3 drops of rennet are then added to the milk and mixed thoroughly. The tube may be put either in an incubator at 37° C. or in warm water at about 40° C. If the tubes can be placed in a slanting position, the curd will settle to one side and clear milk serum, with which the test is made, will separate. Serum will form in about 2 hours and may then be used the same as blood serum in the rapid test described by Huddleson. Tests may be made as soon as the serum separates; but, if the sample can be placed in an ice box or cold place, the fine particles of curd will settle and leave a much clearer serum. Sour milk will give an unreliable test. Milk in which the curd has become partially digested cannot be used as this interferes with the test.

FLAGELLA: Stain for, Farconi, A. Bull. d'histol. appliq. à la physiol. **6:** 306, 1929.

The writer describes the method for staining bacterial flagella proposed by Petragnani in 1922. The first step is mordanting of the carefully prepared bacterial films in a mixture of the following solutions:

Solution A.

Tannic acid (purest)	7.0 gm.
Ferric chlorid	35.0 gm.
Ethyl alcohol	35.0 c.c.
Distilled water	15.0 c.c.

Solution B.

Potassium alum (crystals)	3.0 gm.
Zinc acetate (crystals)	0.5 gm.
Acetic acid, glacial	3.0 drops
Distilled water	10.0 c.c.

After washing, the preparation is stained cold in Ziehl's carbolfuchsin, gentian violet, or in a saturated alcoholic solution of crystal violet in anilin water. (Formulas not given.)

STAIN: Combined Nuclear and Differential, Brilmyer, G. J. Science **68:** 114, 1928.

1. Stain sections in Delafield's hematoxylin for five minutes.
2. Pass through distilled water to remove excess stain.
3. Stain in 0.2 per cent aqueous solution of acid fuchsin for one minute.
4. Pass through distilled water to remove excess stain.
5. Stain in the following solution for two to three hours:

Anilin blue (water soluble)	0.5 gm.
Orange G	2.0 gm.
Phosphomolybdic acid (1 per cent aqueous solution)	100.0 c.c.

6. Pass through distilled water to remove excess stain.
7. Pass successively (rapidly) through the following grades of alcohol: 36 per cent, 70 per cent, and 95 per cent.
8. Complete dehydration in absolute alcohol in one-half to one minute. (Water-free acetone may be used in place of absolute alcohol, with but little or no shrinkage of the cells.)
9. Clear in xylol.
10. Mount with cover glass.

With this staining nuclei will appear a rich red, epithelial cells pink, connective tissue blue and muscle red. Red blood cells will stain yellowish in veins and reddish in arteries. Colloid and mucus stain blue.

The staining seems to follow any fixation well. Sections stained by this method have not faded in five years.

The writer states there is nothing new in the procedure other than the method of combining Delafield's hematoxylin with Mallory's connective tissue stain.

EXUDATES: Classification Text for Exudates and Transudates, D'Allocco, D. Rif. Med. Naples **45:** 1209, 1929.

The following procedure is suggested as a means of differentiating between exudates and transudates:

To 4 or 5 c.c. of alcohol warmed in a test tube to 90° C., the fluid to be examined is allowed to fall drop by drop. Exudates form at once an albuminoid coagulum which settles to the bottom of the tube later rising to the surface. Transudates form a powdery coagulum remaining on the bottom of the tube.

SCOPOMETRY: The Junior Scopometer, Exton, W. G. J. A. M. A. **92:** 708, 1929.

Exton describes a new instrument for the measurement of turbidity and for a new method of colorimetry which uses the extinction point for both determinations.

The instrument is applicable to the determination of sugar in the urine, protein in blood, urine, spinal fluid and various other procedures.

AGGLUTINATION: Stained Slide Microscopic Agglutination Test, Sabin, A. B. Am. J. Pub. Health **19**: 1148, 1929.

The following procedure is applicable to the rapid typing of pneumococci and the determination of antibody:

The procedure for microscopic typing is as follows: One c.c. of a fresh sample of sputum is injected intraperitoneally into a mouse; three to four hours after injection some of the peritoneal fluid is obtained by capillary puncture. A glass slide is marked off into 4 parts, and a minute drop of the peritoneal fluid is expelled upon each one of the 4 partitions. The first is smeared with saline for control, and the others with a loopful of a 1:10 dilution of Type I, of Type II, and of Type III, diagnostic serums respectively. This dilution of serum is chosen largely to eliminate group agglutinins. The smears are made thin so that they dry rapidly; they are then stained for one-half minute with a fuchsin solution (10 c.c. saturated alcoholic solution of basic fuchsin plus 90 c.c. distilled water).

The stain is washed off in running water, and the smears are examined with the oil-immersion lens. If a specific agglutination reaction is observed in one of the smears with diagnostic serum, the organism is of the corresponding type. If no reaction occurs in any of the smears, and pneumococci are clearly seen, a diagnosis of Group IV is made. When it is desired to know whether the organism is one of the fixed types of Group IV, a similar procedure is carried out with the corresponding diagnostic serums. Naturally occurring clumps of organisms differ in appearance from those produced by specific agglutination; they can be recognized by their occurrence in the saline control smear as well. Unless a fresh sample of sputum is used, many of the organisms will have undergone autolysis and therefore more time must be allowed for growth. Since the mouse is not killed, another typing can be done if the first one should show insufficient organisms, and after death of the mouse the type may be confirmed. In the case of Type III, sufficient organisms for a microscopic typing are present as early as two hours after injection. The appearance of the specific reaction with Type III differs from that obtained with other types of pneumococci, primarily on account of the larger size of the capsule.

The technic for carrying out the microscopic antibody test is as follows:

A drop or more of the patient's blood is taken with a capillary. This is either centrifuged or, after coagulation and syneresis, a minute amount of serum is smeared with a loopful of a heat-killed, saline suspension of the type of pneumococci for which it is desired to determine agglutinins. The drops are throughly mixed and smeared very thin, allowed to dry in air, and stained for one-half minute with the fuchsin solution recommended for the microscopic typing test. The specific agglutination reaction is obtained here, but the clumps tend to be smaller.

RECTAL CULTURES: A Simple Method of Making Cultures of the Rectum, Traut, E. F., and Herrold, R. D. J. Infect. Dis. **45**: 172, 1929.

The technic is as follows: Vaseline is sterilized in small individual containers for stock use and finger cots are sterilized by boiling just before use. At first the external surface of the skin and mucous membrane was cleansed throughly with soap and water. Carefully repeated cultures proved this to be unnecessary if the external surface is coated with vaseline before the insertion of the finger. Control cultures were made of the vaseline, fingers cots and external surface of the skin and mucous membrane. Inoculations are made on ascites-blood agar plates and the original inoculum streaked out in the usual way. Cultures have been also without ascites to make certain that the ascites fluid was not bactericidal. No difference could be noted between cultures made on ascites-blood agar and those on plain blood agar. The plates without ascites were made with 1 per cent agar. The more luxuriant growth on the softer mediums is striking regardless of the bacteria implanted.

Massage of the rectum with sterile, vaseline-coated finger cot or glove, followed by inoculation of its tip onto blood agar plates, gave cultures of streptococci and other

bacteria in larger numbers as compared with the colon bacilli than could be secured by making cultures from the feces. The persistence of similar bacteria on repeated culture would indicate that the organisms found are not accidental saprophytes. A comparison of the fermentations by the streptococci isolated on successive culture is further evidence of the reliability of the results. The method offers an easy means of studying the relations of pathogenic bacteria of the rectum to disturbances of the intestinal tract itself, and also to arthritis and other systemic diseases.

ARTHRITIS: The Bacteriology of the Blood and Joints in Chronic Infectious Arthritis, Cecil, R. L., Nichols, E. E., and Stainsby, W. J. Arch. Int. Med. 43: 571, 1929.

The authors present evidence supporting the conception of arthritis as a streptococcal infection.

1. Blood cultures: The technic employed for blood cultures was an adaptation of that recommended by Clawson in his blood culture studies of rheumatic fever.

Twenty cubic centimeters of blood were taken aseptically from the arm with a Luer syringe, placed in two sterile culture tubes and allowed to clot. Each tube was treated separately in the following way:

The tubes was centrifugated, and all the serum drawn off with a sterile pipette. The clot was then broken up in the original culture tube with a sterile glass tube, ¼ inch (6.35 mm.) in diameter. The fragments of clot were drawn up in the same glass tube, and transferred to a 3 ounce bottle containing 50 c.c. of beef heart infusion broth with a P_H of 7.6 (0.5 per cent sodium chloride, 1 per cent peptone). The bottle was then put in the incubator at 37 C. and left there unopened for five days.

At the end of this time a tube containing 8 c.c. of 1.5 per cent beef heart infusion agar was placed in the water-bath and heated until the agar was completely melted. The tube was then partially cooled and 0.5 c.c. of whole rabbit blood added to it. Finally, the tube was seeded with 0.1 c.c. of broth from the original blood culture, and the contents poured into a Petri dish. The culture was allowed to incubate for from twenty-four to forty-eight hours. Similar pour-plate cultures were made every three to five days thereafter until the original blood culture had been in incubation for thirty days If, at the end of this time, the subcultures were still sterile, the sediment in the original blood culture bottle was drawn out with a sterile glass tube and centrifugated. After centrifugating, part of the sediment was examined by means of stained smears, while the remainder was cultured part of it in fresh blood broth and part of it on blood agar plates. If these final cultures from the sediment showed no growth, the blood culture was considered sterile.

All of these cultures and transfers were made under a hood, in order to eliminate contaminations as far as possible. All contaminated cultures were discarded.

When colonies appeared on the plates, they were transferred into blood broth and identified by the usual bacteriologic methods.

2. Joint cultures: Cultures from joints were made in blood broth from synovial fluid or synovial membrane, or from bony curettings removed from the joint at operation. When only tissue was available, the material was put in a bottle containing blood broth and macerated with a glass rod. These cultures, like the blood cultures, were incubated for several weeks and subcultured from time to time.

APPENDICITIS: The Histologic Diagnosis of Chronic, Menon, T. B. Indian J. M. Research 16: 1019, 1929.

In the histologic diagnosis of chronic appendicitis the three features that are of importance are the presence of marked fibrosis and thickening of the submucosa, eosinophile infiltrations of the submucosa and fibrosis or cell infiltrations of the muscle coat.

Each of these by itself is sufficient to warrant a report of chronic appendicitis.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

The Female Sex Hormone[*]

IN THIS volume are detailed the researches of the author begun in 1904 and continued to the present time upon the nature of the female sex hormone.

Part I (142 pages) is concerned with the biologic, pharmacologic, and chemical phases of the subject, while Part II (132 pages) is devoted to a discussion of clinical investigations based upon the female sex hormone blood test, the technic and applications of which are presented in detail.

The volume may be accepted as a full and comprehensive presentation of a subject at present under discussion. A list of 528 references is appended.

Synopsis of The Practice of Preventive Medicine[†]

WHILE this volume is intended primarily for the use of students, it may be read with profit by the practitioner as well.

It is based upon the fact that courses in preventive medicine occupying a separate place in the medical curriculum are usually designed to consider the subject from the standpoint of the community rather than the individual, and so come to be regarded by the student as a subject apart from the clinical branches of medicine.

It may be said, also, that this viewpoint is too often carried over into the practice of medicine whereas every doctor should be an exponent of preventive medicine as applied to the individual.

The whole field of medicine is briefly covered, presenting the composite ideas of the faculty and stressing, under each heading, the prophylactic measures that should help the doctor in protecting the health of his patients. The volume is interleaved for notes and presents a comprehensive survey of the subject, brief but to the point.

The Treatment of Diabetes Mellitus[‡]

THIS is an exceedingly valuable book both for the physician and the diabetic.

It presents in detail the plan of treatment used by Sansum and his collaborators.

The material is presented in two parts: a Medical Section and a Dietetic Section, both of which represent a clear-cut, intelligible discussion of a complicated subject all too often too little understood by both patient and physician.

The volume can be most highly recommended.

Three Minute Medicine[§]

A SERIES of short essays on varied medical subjects intended for popular consumption.

[*]The Female Sex Hormone. By R. T. Frank, Gynecologist, Mt. Sinai Hospital, New York. Cloth, 324 pages, 86 illustrations, 36 graphs, C. C. Thomas, Baltimore, Md.

[†]Synopsis of the Practice of Preventive Medicine, as Applied in the Basic Medical Sciences and Clinical Instruction at the Harvard Medical School. Cloth, 194 pages. Harvard University Press.

[‡]The Treatment of Diabetes Mellitus With Higher Carbohydrate Diets, A Textbook for Physicians and Patients. By W. D. Sansum, P. A. Gray, and R. Bowden. Cloth, 309 pages, numerous tables. Harper & Bros., New York.

[§]Three Minute Medicine, Brief Essays on Popular Medicine. By L. R. Effler, M.D., Cloth, 452 pages. R. G. Badger, Boston.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

The Volume of the Blood and Plasma In Health and Disease[*]

IN THE fifteen years which have elapsed since its introduction by Rowntree, Keith, and Geraghty the dye method of determining the plasma and blood volume has been subjected to extensive study and, in its present state, may be accepted as a valuable clinical aid.

The present monograph from The Mayo Clinic presents a comprehensive and authoritative review of all that is known of this subject to date presented by the authors, not only to clarify the subject, but to emphasize the clinical value of such studies.

A short historical résumé begins the volume followed by a discussion of the criticisms which have been made of the dye method, some valid and others not, and this in turn by a detailed description of the proper technic for its performance.

The normal values are then discussed. These, it would appear lie between 40 and 60 c.c. of plasma per kilogram regardless of sex. Three new terms are suggested: "normovolemia" (within the normal mean); "hypervolemia" (increased blood volume); and "hypovolemia" (decreased blood volume).

The remainder of the volume discusses the findings encountered in various conditions; obesity, diseases of the blood; diseases of the spleen and liver; various types of edema, diseases of the vascular system; diseases of the endocrine glands; and miscellaneous conditions. The clinical data is further discussed in a separate chapter.

In hypertension an increased blood volume does not seem to be of importance and, despite the hypertension in glomerulonephritis, the blood volume is decreased.

The outstanding finding is the vigorous effort made by the organism to maintain the plasma volume within narrow constant limits. It is, therefore, one of the most striking and significant constants of the body. While its true relation to and significance to disease remain to be ascertained, the present volume constitutes a definite and valuable contribution to the subject and well repays study.

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[*]The Volume of the Blood and Plama in Health and Disease. By Leonard G. Rowntree, M.D., and George E. Brown, M.D., Division of Medicine, The Mayo Clinic and The Mayo Foundation, Rochester, Minn., with the technical assistance of Grace M. Roth. Cloth, 219 pages, illustrated. Philadelphia & London: W. B. Saunders Co. 1929.

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EDITORIALS

The Backgrounds of Biologic Therapy

IT IS a long step from the first report of Edward Jenner in 1798 on his results from cowpox vaccination, and from the studies of Pasteur in 1884 on the prevention of rabies, to our present-day immunologic understanding and practice. Were these two pioneers to revisit the world they would undoubtedly express the greatest astonishment at the tremendous advances that have been made.

And yet the question has been seriously raised of late, and quite rightly, whether our forward strides have been especially great, particularly when measured in terms of the clinical application of today's generally accepted immunologic principles.

Hektoen and Irons have tabulated the replies to a questionnaire on vaccine therapy which was answered by 1,261 practicing physicians, and of this number only seventeen physicians consider vaccine therapy to be a generally useful and superior method of treating infectious diseases. Four hundred and

thirty have never used autogenous vaccines in the treatment of any disease, 142 have used them rarely, and 172 report having abandoned the use of autogenous vaccines in therapy. Five hundred and seventy-seven did not use or have never used stock polyvalent vaccines in the treatment of any disease; 49 have used them very rarely; 198 report having abandoned the use of stock polyvalent vaccines because of their failure as therapeutic agents.

The authors point out that from 1906, the date of Wright's introduction of vaccine treatment, the number of papers on vaccines in medical journals constantly increased until it reached a formidable volume around 1912. Since then interest in the treatment has evidently declined as measured by the number of contributions in current literature.

Manwaring and Krueger calculate that our fundamental immunologic theories and current experimental laboratory methods have assayed less than 5 per cent clinically verifiable truth. Applying our present knowledge it would appear that of twenty theoretically logical laboratory-endorsed vaccines and antiserums, only one would prove a clinical success.

The two preceding references constitute ample evidence that it is high time that we take inventory. At the same time, there is great danger of these writers being misquoted and misunderstood. Neither group has denied the remarkable advances that have been made in this field. Hektoen and Irons specifically state that they do not draw conclusions with regard to the value or lack of value of vaccines in treatment. Their one interest has been to ascertain what opinion practicing physicians have formed of this method and how that opinion has affected the present use of vaccines. Indeed, if they had discovered enthusiastic proponents of vaccine therapy in some of the diseases which they tabulate such as appendicitis, diabetes, nephritis, dermatitis, eczema, focal infection, gall bladder infection, gastric ulcer, hay fever, meningitis, peritonitis, and epilepsy, we would be forced to conclude that the physician of today was using extremely poor judgment in his clinical application of our immunologic theories.

Manwaring and Krueger, far from being discouraged, remark that modern immunology is justly proud of its 5 per cent clinical efficieney, the high watermark of all history. One need only recall by way of comparison the 2 per cent verifiable clinical value of the fundamental biologic theories of the mid-Victorian era and the 1 per cent clinical coefficient of Aesculapius.

However, it is really a question whether the average physician is as critical in his actual practice as he is in his replies to questionnaires. As Kinsella remarks, one has only to compare the catalogue of any prominent drug company of twenty years ago with their most recent list to realize that the treatment of infectious diseases has been changed from one in which drugs played the most important part to one in which such biologic products as serums and vaccines have assumed a great prominence.

"This condition reflects an attitude on the part of the physician which is due to the lack of uniform teaching regarding the nature of infection, its etiology and the means by which an infectious disease may be neutralized. The medical student hears contradictory evidence in many fields of bacteriology. At the same time the information which he receives concerning anti-

gens and antibodies is so rigidly definite that he leans to a belief in specificity and is inclined to accept as specific the various agents for immunization, however loosely supported they may be by the uncontrolled observations of clinicians or the claims of salesmen."

What have we to show for the 5 per cent clinically efficient immunologic remedies? Smallpox vaccine, rabies vaccine, typhoid vaccine, diphtheria toxin-antitoxin, diphtheria antitoxin, tetanus antitoxin, antipneumococcus serum Type I, antimeningococcus serum, streptococcus scarlatinae antitoxin, poliomyelitis immune serum, possibly erysipelas antitoxin, antivenin, pollen and other allergenic extracts, the Wassermann test, the Widal test, and similar precipitin and agglutination tests. Remove all of these and the armamentarium of the physician would be sadly depleted. The modern specific treatment of the allergic diseases is as directly the outgrowth of our present understanding of the phenomena of anaphylaxis and immunity as is the case with any other biologic measure employed in modern medicine. And 66 per cent of relief in hay fever, 50 per cent in allergic eczema, and 37 per cent in migraine by specific measures only, are truly substantial figures.

The concepts of a hundred years hence will no more resemble those of today than do our present concepts resemble those of Galen which controlled medicine for 800 years. And yet the wonder is that we have been able to accomplish so much by the clinical application of what little today is known in the field of immunology. Our indebtedness to the pioneers of the last half century is great indeed. Let us glance back for a moment at the historical background in one section of this broad field.

ANAPHYLAXIS

As early as 1839 Magendie had noticed that rabbits which had received without untoward symptoms a first injection of albumin could not sustain the same dose several days later. In 1888 Victor C. Vaughan observed that laboratory animals which had once been inoculated could not safely be used for subsequent experimentation. In the standardization of diphtheria antitoxin it soon became evident that guinea pigs that survived one test could not be relied upon in a second one. In the late nineties Parke-Davis and Company, aware of this fact, offered to supply the Hygienic Laboratory of the University of Michigan with "used pigs" at a small price. The offer was accepted but the animals were found to be high at any price, as they suddenly and unexplainably died when used in experimental work.

Undoubtedly many observers of that time were aware of this unusual manifestation, for several years later Theobald Smith mentioned the phenomenon to Ehrlich, who set his student Otto to work to find the explanation. Otto in publishing his findings described this phenomenon as the ***Theobald Smith Phenomenon.***

Flexner in 1894 observed that rabbits which had received dog serum without untoward effect died promptly after a second dose several days or weeks later. The second dose was often smaller than the first. Behring at about the same time (1893) also observed the curious results of reinjection of

diphtheria antitoxin in the guinea pig. Working with him Knorr and Kitasato determined that the second dose might be fatal even though it was seven or eight hundred times smaller than the initial dose. Behring described this as a paradoxical reaction.

In 1894, again, Arloing and Courmont observed that successive injections of donkey serum into man produced increasingly toxic effects. Richet in 1898 while studying the effects of eel serum on dogs often observed death after the second or third injection. Courmont noticed in 1900 that successive small doses of tuberculous pleural exudate caused guinea pigs to die before having received a quarter of the total dose which they could easily withstand in a single injection.

Thus we see that between 1839 and 1902 the phenomenon that we now designate anaphylaxis had been observed often enough, but no attempt had been made to study it farther or to explain it, recourse merely being had to such terms as atypical or paradoxic reactions. It remained for Richet and Portier to make a really systematic study of this phenomenon and to offer a tentative explanation. Their communication was first published on February 15, 1902, and it was on this day that allergy or anaphylaxis as we understand it now, first saw the light of day.

Richet and Portier who were yatching as guests of the Prince of Monaco in the southern seas, inaugurated, at the latter's suggestion, a study of the toxic properties of the *physalia*, commonly known as Portuguese Man o'War. These like our own jelly fish produce an intense skin irritation on contact. On their return to France they were unable to obtain a further supply of *physalia*, so they determined to carry on their experiments with the *sea anemone*.

Making glycerine extracts of the tentacles, they attempted to determine the toxic dose of the urticating substance. Just as with diphtheria antitoxin, dogs that survived were held over for later testing.

It was a dog which answered to the name of Neptune whose contribution to humanity was the opening of the portals of the science of allergy. Neptune, an unusually healthy and robust dog received 0.1 c.c. of glycerine extract of *anemone* hypodermically, without becoming ill. Twenty-two days later while still in the best of health Neptune received another dose of 0.1 c.c. A few seconds later he became extremely ill, with extreme asthenia, dyspnea, diarrhea and bloody vomiting. He soon became unconscious and was dead within twenty-five minutes.

Richet's subsequent studies and interpretations as summarized in his theory of anaphylaxis, published in 1902, presented an entirely new understanding of these paradoxical reactions. The keynote to the phenomenon lay in two facts: first, that a substance which an animal could receive with impunity might on reinoculation become most highly poisonous; and second that a certain amount of time must elapse between the two inoculations for the toxic change to become manifest.

Space does not permit a review of Richet's theory. Suffice it to say that evidence was presented that the introduction of a foreign protein even in

small quantity into a living animal produced profound changes in the tissues of the inoculated animal.

This observation was at once utilized to explain the tuberculin skin reaction which had been described by Koch in 1890. The tuberculin reaction was now accepted as evidence that a profound change had occurred in the tissue of the human organism as a result of the presence of the tubercle bacillus protein.

Victor C. Vaughan in the meantime had been carrying on research into the nature of bacteria and bacterial infection. As early as 1890 he had demonstrated that the bacterial cell contains or metabolizes a poisonous substance and that this substance is the same or similar in different types of bacteria. Following Richet's announcement he at once applied these observations to his studies of bacterial infection and was able to confirm Richet's findings. He next demonstrated that the poisonous fraction which he was able to separate from the protein molecule appeared quite the same or similar in such diverse proteins as bacteria, horse serum and egg albumin. On the basis of this and further observations he evolved his own theory of protein sensitization which was presented in its completed form in 1906. He applied this knowledge in the development of his theory of bacterial infection and immunity.

Richet proposed the term anaphylaxis, meaning "without protection," as an interpretation of his observation that animals in whom he was building up protection by injections sometimes apparently lost this protection completely, becoming abnormally sensitive. Vaughan and Wheeler demonstrated that the same fundamental process was at work, both in immunity and in anaphylaxis, and to this extent the term anaphylaxis is a misnomer. Today we might with equal reason use the term hyperphylaxis for the same phenomenon. Von Pirquet proposed the more acceptable term, allergy, in 1906, meaning altered energy, or altered reactivity, expressive of the deep-seated systemic changes which have taken place within the organism following a first introduction of foreign protein. It is this term which has been adopted in clinical work. Anaphylaxis is still used in clinical medicine but usually to express that fulminating reaction occasionally observed after parenteral inoculation, which presents a picture altogether similar to that seen in experimental animals and is known generally as anaphylactic shock.

Many other theories of anaphylaxis have been proposed but it is safe to say that subsequent developments and our present knowledge are founded on three theories, the Ehrlich side chain theory from Germany, the Richet anaphylaxis theory from France, and the Vaughan protein poison theory from America.

These three theories were offered to explain phenomena that were known to exist at that time. Further laboratory investigations have produced many phenomena which cannot be satisfactorily explained by any one or all three of the theories, and which probably eventually will call for their reconstruction or discard. However, in the meantime we have traveled surprisingly far especially in the field of clinical allergy with the aid of these hypotheses.

As Victor Vaughan remarked in 1893, "The value of a theory does not wholly depend upon its truth, but is rather to be measured by the fruitfulness of the lines of investigation that it opens. Indeed a theory may be wholly erroneous and yet it may lead to most important discoveries."

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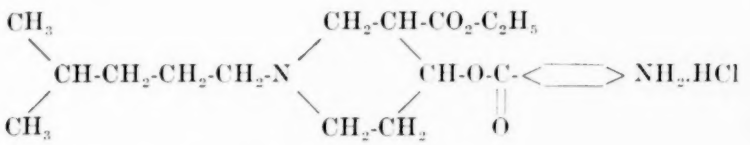
Kinsella, Ralph A.: The Clinical Value of Serums and Vaccines, J. A. M. A. **93**: 1524, November 16, 1929.

—*W. T. V.*

Erratum

In the article *Studies in the Pharmacology of Local Anesthetics II*, by Harold W. Coles and C. L. Rose, December, 1929, on page 240, Formula No. 0 should be written as



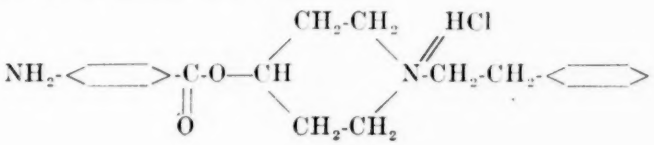
Chemical name for No. 33-G should be written as

Gamma-(2-methyl piperidino)-propyl benzoate HCl.

On page 242, one of the hydrogens should be removed from the beta-carbon of formula No. 56 to which the methyl group is joined.

Formulas Nos. 92 and 93 should have double bonds between the two CH groups.

Formula No. 58 should be written as



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News and Notes

The Ninth Annual Convention of the American Society of Clinical Pathologists will be held in Detroit, Michigan, June 20, 21, and 23, 1930. The Book Cadillac Hotel has been selected as our official headquarters and the management assures us that all reservations if so desired will be carried on through the meeting of the American Medical Association the following week. You are, however, urged to make your reservations as early as possible in order that you may secure the exact accommodations you want.

The scientific meetings of the Society will be held in the Crystal Ballroom of the Book Cadillac Hotel with the scientific and commercial exhibits in the adjoining Italian Garden.

Fellows of the American Society of Clinical Pathologists are invited to reserve space for a scientific exhibit at Convention and to enter the contest. A Gold and a Silver Medal will be awarded to the members presenting the best scientific exhibits. This should be a stimulus for a very interesting and instructive display.

If you wish to present a scientific paper you may communicate with the Secretary, Dr. H. J. Corper, 256 Metropolitan Building, Denver Colorado, giving the exact title and, if possible, an abstract.

Reservations for hotel accommodations and space in the scientific exhibit may also be made through the Secretary.

The plans made by the Local Committee on Arrangements under the Chairmanship of Dr. F. W. Hartman insure a very successful and enjoyable convention for all those in attendance. Lock up your workshop and attend the Detroit Convention to procure your annual scientific stimulant.